FIG. 1

sequence_AA_2D12.5_variable domains.txt

>2D12.5VL_MOUSE
(1) QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGNNNRPPGVPARFSGSLIGDKAALTIAGTQTED
EAIYFCALWYSNHWVFGGGTRLTVLG

(2) CDR1 - RSSTGAVTTSNYAN
(3) CDR2 - GNNNRPP
(4) CDR3 - ALWYSNHWV

>2D12.5VH_MOUSE
(5) QVKLQESGPGLVQPSQSLSITCTVSGFSLTDYGVHWVRQSPGKGLEWLGVIWSGGGTAYTAAFISRLNIY
KDNSKNQVFFEMNSLQANDTAMYYCARRGSYPYNYFDVWGQGTTVTVSS

(6) CDR1 - DYGVH
(7) CDR2 - VIWSGGGTAYTAAFIS
(8) CDR3 - RGSYPYNYFDV

FIG. 2

Translation of 2D12.5 VH variable genes

```
                                        10        20        30        40        50
                                ....|....|....|....|....|....|....|....|....|....|
(9) 2d12.5 VH native hybridoma   1  VKLQESGPGLVQPSQSLSITCTVSGFSLTDYGVHWVRQSPGKGLEWLGVI  50
(10)2d12.5 VH native cloned      1  ..................................................  50
(11)2d12.5 VH N87D_cloned        1  ..................................................  50
(12)2d12.5 VH N87D_G53C_cloned   1  ..................................................  50
(13)2d12.5 VH_N87D_G54C_cloned   1  ..................................................  50
(14)2d12.5 VH N87D_G55C_cloned   1  ..................................................  50

                                        60        70        80        90       100
                                ....|....|....|....|....|....|....|....|....|....|
2d12.5 VH native hybridoma      51  WSGGGTAYTAAFISRLNIYKDNSKNQVFFEMNSLQANDTAMYYCARRGSY  100
2d12.5 VH native cloned         51  ..................................................  100
2d12.5 VH N87D_cloned           51  ..............................D...................  100
2d12.5 VH N87D_G53C_cloned      51  ..C...........................D...................  100
2d12.5 VH_N87D_G54C_cloned      51  ...C..........................D...................  100
2d12.5 VH N87D_G55C_cloned      51  ....C........................D...................  100

                                        110
                                ....|....|....|...
2d12.5 VH native hybridoma      101 PYNYFDVWGQGTTVTVSS  118
2d12.5 VH native cloned         101 ................A  118
2d12.5 VH N87D_cloned           101 ................A  118
2d12.5 VH N87D_G53C_cloned      101 ................A  118
2d12.5 VH_N87D_G54C_cloned      101 ................A  118
2d12.5 VH N87D_G55C_cloned      101 ................A  118
```

FIG. 3A

2D12.5 VH variable genes

```
                                                  10        20        30        40        50
                                                  ....|....|....|....|....|....|....|....|....|....|
(15) 2d12.5 VH nativ  hybridoma   1   GTGAAGCTGCAGGAGTCAGGACCTGGCCTAGTGCAGCCCTCACAGAGCCT  50
(16) 2d12.5 VH native cloned      1   ................T.................................... 50
(17) 2d12.5 VH N87D_cloned        1   ................T.................................... 50
(18) 2d12.5 VH N87D_G53C_cloned   1   ................T.................................... 50
(19) 2d12.5 VH_N87D_G54C_cloned   1   ................T..G................................. 50
(20) 2d12.5 VH N87D_G55C_cloned   1   ................T.................................... 50

                                                  60        70        80        90        100
                                                  ....|....|....|....|....|....|....|....|....|....|
2d12.5 VH native hybridoma        51  GTCCATCACCTGCACGGTCTCTGGTTTCTCATTAACTGACTATGGTGTAC 100
2d12.5 VH native cloned           51  .................................................... 100
2d12.5 VH N87D_cloned             51  .................................................... 100
2d12.5 VH N87D_G53C_cloned        51  .................................................... 100
2d12.5 VH_N87D_G54C_cloned        51  .................................................... 100
2d12.5 VH N87D_G55C_cloned        51  .................................................... 100

                                                  110       120       130       140       150
                                                  ....|....|....|....|....|....|....|....|....|....|
2d12.5 VH native hybridoma        101 ACTGGGTTCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGAGTGATA 150
2d12.5 VH native cloned           101 .................................................... 150
2d12.5 VH N87D_cloned             101 .................................................... 150
2d12.5 VH N87D_G53C_cloned        101 .................................................... 150
2d12.5 VH_N87D_G54C_cloned        101 .................................................... 150
2d12.5 VH N87D_G55C_cloned        101 .................................................... 150

                                                  160       170       180       190       200
                                                  ....|....|....|....|....|....|....|....|....|....|
2d12.5 VH native hybridoma        151 TGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTCATATCCAGACTGAA 200
2d12.5 VH native cloned           151 .................................................... 200
2d12.5 VH N87D_cloned             151 .................................................... 200
2d12.5 VH N87D_G53C_cloned        151 ......T............................................. 200
2d12.5 VH_N87D_G54C_cloned        151 .........T.T........................................ 200
2d12.5 VH N87D_G55C_cloned        151 ............T....................................... 200

                                                  210       220       230       240       250
                                                  ....|....|....|....|....|....|....|....|....|....|
2d12.5 VH native hybridoma        201 CATCTACAAGGACAATTCCAAGAACCAAGTTTTCTTTGAAATGAACAGTC 250
2d12.5 VH native cloned           201 .................................................... 250
2d12.5 VH N87D_cloned             201 .................................................... 250
2d12.5 VH N87D_G53C_cloned        201 .................................................... 250
2d12.5 VH_N87D_G54C_cloned        201 .................................................... 250
2d12.5 VH N87D_G55C_cloned        201 .................................................... 250

                                                  260       270       280       290       300
                                                  ....|....|....|....|....|....|....|....|....|....|
2d12.5 VH native hybridoma        251 TGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGTAGCTAC 300
2d12.5 VH native cloned           251 .................................................... 300
2d12.5 VH N87D_cloned             251 ........G........................................... 300
2d12.5 VH N87D_G53C_cloned        251 ........G........................................... 300
2d12.5 VH_N87D_G54C_cloned        251 ........G........................................... 300
2d12.5 VH N87D_G55C_cloned        251 ........G........................................... 300

                                                  310       320       330       340       350
                                                  ....|....|....|....|....|....|....|....|....|....|
2d12.5 VH native hybridoma        301 CCTTACAACTACTTCGATGTCTGGGGCCAAGGGACCACAGTCACCGTCTC 350
2d12.5 VH native cloned           301 ....................................G........... 350
2d12.5 VH N87D_cloned             301 ....................................G........... 350
2d12.5 VH N87D_G53C_cloned        301 ....................................G........... 350
2d12.5 VH_N87D_G54C_cloned        301 ....................................G........... 350
2d12.5 VH N87D_G55C_cloned        301 ....................................G........... 350
```

FIG. 3B

2D12.5 VH variable gen s

		....	
2d12.5 VH native hybridoma	351	CTCA	354
2d12.5 VH native cloned	351	.G..	354
2d12.5 VH N87D_cloned	351	.G..	354
2d12.5 VH N87D_G53C_cloned	351	.G..	354
2d12.5 VH_N87D_G54C_cloned	351	.G..	354
2d12.5 VH N87D_G55C_cloned	351	.G..	354

FIG. 4

Translation of 2D12.5 VL genes

```
                                        10        20        30        40        50
                                        ....|....|....|....|....|....|....|....|....|....|
(21) 2d12.5 VL native hybridoma      1  AVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIG  50
(22) 2d12.5 VL_native cloned         1  ..................................................  50
(23) 2d12.5 VL N53C_cloned           1  ..................................................  50

                                        60        70        80        90        100
                                        ....|....|....|....|....|....|....|....|....|....|
     2d12.5 VL native hybridoma     51  GNNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWVFG  100
     2d12.5 VL_native cloned        51  ..................................................  100
     2d12.5 VL N53C_cloned          51  .C................................................  100

                                        ....|....
     2d12.5 VL native hybridoma    101  GGTRLTVLG  109
     2d12.5 VL_native cloned       101  ...K....S  109
     2d12.5 VL N53C_cloned         101  ...K....S  109
```

FIG. 5

2D12.5 VL variable genes

```
                                        10        20        30        40        50
                                        ....|....|....|....|....|....|....|....|....|....|
(24) 2d12.5 VL native hybridoma     1   GCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGT  50
(25) 2d12.5 VL_native cloned        1   ..................................................  50
(26) 2d12.5 VL N53C_cloned          1   ..................................................  50

                                        60        70        80        90        100
                                        ....|....|....|....|....|....|....|....|....|....|
2d12.5 VL native hybridoma         51   CACACTCACTTGTCGCTCAAGTACTGGGGCTGTTACGACTAGTAACTATG 100
2d12.5 VL_native cloned            51   .................................................. 100
2d12.5 VL N53C_cloned              51   .................................................. 100

                                       110       120       130       140       150
                                        ....|....|....|....|....|....|....|....|....|....|
2d12.5 VL native hybridoma        101   CCAACTGGGTCCAAGAGAAACCAGATCATTTATTCACTGGTCTAATAGGT 150
2d12.5 VL_native cloned           101   .................................................. 150
2d12.5 VL N53C_cloned             101   .................................................. 150

                                       160       170       180       190       200
                                        ....|....|....|....|....|....|....|....|....|....|
2d12.5 VL native hybridoma        151   GGTAATAATAACCGACCTCCAGGTGTTCCTGCCAGATTCTCAGGCTCCCT 200
2d12.5 VL_native cloned           151   .................................................. 200
2d12.5 VL N53C_cloned             151   ...TG.............................................. 200

                                       210       220       230       240       250
                                        ....|....|....|....|....|....|....|....|....|....|
2d12.5 VL native hybridoma        201   GATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTGAGGATG 250
2d12.5 VL_native cloned           201   .................................................. 250
2d12.5 VL N53C_cloned             201   .................................................. 250

                                       260       270       280       290       300
                                        ....|....|....|....|....|....|....|....|....|....|
2d12.5 VL native hybridoma        251   AGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTGTTCGGT 300
2d12.5 VL_native cloned           251   .................................................. 300
2d12.5 VL N53C_cloned             251   .................................................. 300

                                       310       320
                                        ....|....|....|....|....|..
2d12.5 VL native hybridoma        301   GGAGGAACCAGACTGACTGTCCTAGGC 327
2d12.5 VL_native cloned           301   ..G.......A.............A.. 327
2d12.5 VL N53C_cloned             301   ..G.......A.............A.. 327
```

FIG. 6

Translation of Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

```
                                    10        20        30        40        50
                                    ....|....|....|....|....|....|....|....|....|....|
(27) 2dVL-TTCL native_cloned        1   RSAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGL 50
(28) 2dVL-TTCL N53C_cloned          1   .................................................. 50
(29) 2d12.5 VL native hybridoma     1   --................................................ 48
(30) TTCL template for gene assembl 1   ---------------------------------------------- 1

                                    60        70        80        90        100
                                    ....|....|....|....|....|....|....|....|....|....|
     2dVL-TTCL native_cloned        51  IGGNNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWV 10
     2dVL-TTCL N53C_cloned          51  ...C.............................................. 10
     2d12.5 VL native hybridoma     49  ................................................. 9
     TTCL t mplate for gene assembl 1   ---------------------------------------------- 1

                                    110       120       130       140       150
                                    ....|....|....|....|....|....|....|....|....|....|
     2dVL-TTCL native_cloned        101 FGGGTKLTVLSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV 15
     2dVL-TTCL N53C cloned          101 .................................................. 15
     2d12.5 VL native hybridoma     99  .....R....G                                        10
     TTCL template for gene assembl 1   ----------.......................................... 39

                                    160       170       180       190       200
                                    ....|....|....|....|....|....|....|....|....|....|
     2dVL-TTCL native_cloned        151 QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV 2c
     2dVL-TTCL N53C_cloned          151 .................................................. 2a
     2d12.5 VL native hybridoma
     TTCL template for gene assembl 40  .................................................. 8

                                    210       220
                                    ....|....|....|....|.
     2dVL-TTCL native_cloned        201 THQGLSLPVTKSFNRGEC*F*  221
     2dVL-TTCL N53C_cloned          201 ..................*.*  221
     2d12.5 VL native hybridoma
     TTCL template for gene assembl 90  .................     107
```

FIG. 7A

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

```
                                    10        20        30        40        50
                                    ....|....|....|....|....|....|....|....|....|....|
(31) 2dVL-TTCL native_cloned        1   AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGA 50
(32) 2dVL-TTCL N53C_clon d          1   .................................................. 50
(33) 2d12.5 VL native hybridoma     1   ----------..................................... 44
(34) TTCL template for gene assem   1   ---------------------------------------------- 1

                                    60        70        80        90        100
                                    ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned             51  AACAGTCACACTCACTTGTCGCTCAAGTACTGGGGCTGTTACGACTAGTA 10(
2dVL-TTCL N53C_cloned               51  .................................................. 10(
2d12.5 VL native hybridoma          45  .................................................. 94
TTCL template for gene assem        1   ---------------------------------------------- 1

                                    110       120       130       140       150
                                    ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned             101 ACTATGCCAACTGGGTCCAAGAGAAACCAGATCATTTATTCACTGGTCTA 15(
2dVL-TTCL N53C_cloned               101 .................................................. 15(
2d12.5 VL native hybridoma          95  .................................................. 14
TTCL template for gene assem        1   ---------------------------------------------- 1

                                    160       170       180       190       200
                                    ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned             151 ATAGGTGGTAATAATAACCGACCTCCAGGTGTTCCTGCCAGATTCTCAGG 20
2dVL-TTCL N53C_cloned               151 .........TG.................................... 20
2d12.5 VL native hybridoma          145 .................................................. 19
TTCL template for gene assem        1   ---------------------------------------------- 1

                                    210       220       230       240       250
                                    ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned             201 CTCCCTGATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTG 25
2dVL-TTCL N53C_cloned               201 .................................................. 25
2d12.5 VL native hybridoma          195 .................................................. 24
TTCL template for gene assem        1   ---------------------------------------------- 1

                                    260       270       280       290       300
                                    ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned             251 AGGATGAGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTG 3(
2dVL-TTCL N53C_cloned               251 .................................................. 3(
2d12.5 VL native hybridoma          245 .................................................. 2
TTCL template for gene assem        1   ---------------------------------------------- 1

                                    310       320       330       340       350
                                    ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned             301 TTCGGTGGGGGAACCAAACTGACTGTCCTAAGCCGAACTGTGGCTGCACC 3
2dVL-TTCL N53C_cloned               301 .................................................. 3
2d12.5 VL native hybridoma          295 ........A.......G.............G..                  3
TTCL template for gene assem        1   ----------------------------------.............. 1

                                    360       370       380       390       400
                                    ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned             351 ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG 4
2dVL-TTCL N53C_cloned               351 .................................................. 4
2d12.5 VL native hybridoma
TTCL template for gene assem        18  .................................................. (

                                    410       420       430       440       450
                                    ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_clon d             401 CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA (
2dVL-TTCL N53C_clon d               401 ..................................................
2d12.5 VL native hybridoma
TTCL template for gene assem        68  ..................................................
```

FIG. 7B

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

```
                                      460        470        480        490        500
                                      ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned          451  CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGT  50(
2dVL-TTCL N53C_cloned            451  ..................................................  50(
2d12.5 VL native hybridoma
TTCL template for gene assem     118  ..................................................  16

                                      510        520        530        540        550
                                      ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned          501  CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA  55(
2dVL-TTCL N53C_cloned            501  ..................................................  55(
2d12.5 VL native hybridoma
TTCL template for gene assem     168  ..................................................  21

                                      560        570        580        590        600
                                      ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned          551  CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC  60
2dVL-TTCL N53C_cloned            551  ..................................................  60
2d12.5 VL native hybridoma
TTCL template for gene assem     218  ..................................................  26

                                      610        620        630        640        650
                                      ....|....|....|....|....|....|....|....|....|....|
2dVL-TTCL native_cloned          601  ACCCATCAGGGCCTGAGCTTGCCCGTCACAAAGAGCTTCAACAGGGGAGA  65
2dVL-TTCL N53C_cloned            601  ..........T.......................................  65
2d12.5 VL native hybridoma
TTCL template for gene assem     268  ..................................................  31

                                      660
                                      ....|....|....
2dVL-TTCL native_cloned          651  GTGTTAATTCTAGA  664
2dVL-TTCL N53C_cloned            651  ..............  664
2d12.5 VL native hybridoma
TTCL template for gene assem     318  .....           322
```

FIG. 8

Translation of Mous 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

```
                                    10        20        30        40        50
                                    ....|....|....|....|....|....|....|....|....|....|
(35) 2dVH-TTCH_native cloned        1   RSVKLQESGPGLVQPSQSLSITCTVSGFSLTDYGVHWVRQSPGKGLEWLG 50
(36) 2dVH-TTCH_N87D_cloned          1   .................................................. 50
(37) 2dVH-TTCH N87D_G53C_cloned     1   .................................................. 50
(38) 2dVH-TTCH_N87D_G54C_cloned     1   .................................................. 50
(39) 2dVH-TTCH N87D_G55C_cloned     1   .................................................. 50
(40) 2dVH-TTCH expected sequence    1   .................................................. 50
(41) 2d12.5 VH native hybridoma     1   -................................................. 48

                                    60        70        80        90        100
                                    ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned             51  VIWSGGGTAYTAAFISRLNIYKDNSKNQVFFEMNSLQANDTAMYYCARRG 100
2dVH-TTCH_N87D_cloned               51  ..............................D.......... 100
2dVH-TTCH N87D_G53C_cloned          51  ....C.........................D.......... 100
2dVH-TTCH_N87D_G54C_cloned          51  .....C........................D.......... 100
2dVH-TTCH N87D_G55C_cloned          51  ......C.......................D.......... 100
2dVH-TTCH expected sequence         51  .................................................. 100
2d12.5 VH native hybridoma          49  .................................................. 98

                                    110       120       130       140       150
                                    ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned             101 SYPYNYFDVWGQGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVK 150
2dVH-TTCH_N87D_cloned               101 .................................................. 150
2dVH-TTCH N87D_G53C_cloned          101 .................................................. 150
2dVH-TTCH_N87D_G54C_cloned          101 .................................................. 150
2dVH-TTCH N87D_G55C_cloned          101 .................................................. 150
2dVH-TTCH expected sequence         101 .................................................. 150
2d12.5 VH native hybridoma          99  ..................S                               118

                                    160       170       180       190       200
                                    ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned             151 DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT 200
2dVH-TTCH_N87D_cloned               151 .................................................. 200
2dVH-TTCH N87D_G53C_cloned          151 .................................................. 200
2dVH-TTCH_N87D_G54C_cloned          151 .................................................. 200
2dVH-TTCH N87D_G55C_cloned          151 .................................................. 200
2dVH-TTCH expected sequence         151 .................................................. 200
2d12.5 VH native hybridoma

                                    210       220
                                    ....|....|....|....|....|..
2dVH-TTCH_native cloned             201 YICNVNHKPSNTKVDKKAEPKSCDKSR 227
2dVH-TTCH_N87D_cloned               201 ........................... 227
2dVH-TTCH N87D_G53C_cloned          201 ........................... 227
2dVH-TTCH_N87D_G54C_cloned          201 ........................... 227
2dVH-TTCH N87D_G55C_cloned          201 ........................... 227
2dVH-TTCH expected sequence         201 ........................... 227
2d12.5 VH native hybridoma
```

FIG. 9A

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

```
                                        10        20        30        40        50
                                ....|....|....|....|....|....|....|....|....|....|
(42) 2dVH-TTCH_native cloned     1  AGATCTGTGAAGCTGCAGGAGTCTGGACCTGGCCTAGTGCAGCCCTCACA 50
(43) 2dVH-TTCH_N87D_cloned       1  .................................................. 50
(44) 2dVH-TTCH N87D_G53C_cloned  1  .................................................. 50
(45) 2dVH-TTCH_N87D_G54C_cloned  1  ........................G......................... 50
(46) 2dVH-TTCH N87D_G55C_cloned  1  .................................................. 50
(47) 2dVH-TTCH expected sequence 1  .................................................. 50
(48) 2d12.5 VH native hybridoma  1  ................A................................. 44

                                        60        70        80        90       100
                                ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned         51  GAGCCTGTCCATCACCTGCACGGTCTCTGGTTTCTCATTAACTGACTATG 100
2dVH-TTCH_N87D_cloned           51  .................................................. 100
2dVH-TTCH N87D_G53C_cloned      51  .................................................. 100
2dVH-TTCH_N87D_G54C_cloned      51  .................................................. 100
2dVH-TTCH N87D_G55C_cloned      51  .................................................. 100
2dVH-TTCH expected sequence     51  .................................................. 100
2d12.5 VH native hybridoma      45  .................................................. 94

                                       110       120       130       140       150
                                ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned        101  GTGTACACTGGGTTCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGA 150
2dVH-TTCH_N87D_cloned          101  .................................................. 150
2dVH-TTCH N87D_G53C_cloned     101  .................................................. 150
2dVH-TTCH_N87D_G54C_cloned     101  .................................................. 150
2dVH-TTCH N87D_G55C_cloned     101  .................................................. 150
2dVH-TTCH expected sequence    101  .................................................. 150
2d12.5 VH native hybridoma      95  .................................................. 144

                                       160       170       180       190       200
                                ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned        151  GTGATATGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTCATATCCAG 200
2dVH-TTCH_N87D_cloned          151  .................................................. 200
2dVH-TTCH N87D_G53C_cloned     151  ............T......................................... 200
2dVH-TTCH_N87D_G54C_cloned     151  .............T.T..................................... 200
2dVH-TTCH N87D_G55C_cloned     151  ..................T................................. 200
2dVH-TTCH expected sequence    151  .................................................. 200
2d12.5 VH native hybridoma     145  .................................................. 194

                                       210       220       230       240       250
                                ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned        201  ACTGAACATCTACAAGGACAATTCCAAGAACCAAGTTTTCTTTGAAATGA 250
2dVH-TTCH_N87D_cloned          201  .................................................. 250
2dVH-TTCH N87D_G53C_cloned     201  .................................................. 250
2dVH-TTCH_N87D_G54C_cloned     201  .................................................. 250
2dVH-TTCH N87D_G55C_cloned     201  .................................................. 250
2dVH-TTCH expected sequence    201  .................................................. 250
2d12.5 VH native hybridoma     195  .................................................. 244

                                       260       270       280       290       300
                                ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned        251  ACAGTCTGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGT 300
2dVH-TTCH_N87D_cloned          251  .............G.................................... 300
2dVH-TTCH N87D_G53C_cloned     251  .............G.................................... 300
2dVH-TTCH_N87D_G54C_cloned     251  .............G.................................... 300
2dVH-TTCH N87D_G55C_cloned     251  .............G.................................... 300
2dVH-TTCH expected sequence    251  .................................................. 300
2d12.5 VH native hybridoma     245  .................................................. 294

                                       310       320       330       340       350
                                ....|....|....|....|....|....|....|....|....|....|
```

FIG. 9B

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

```
2dVH-TTCH_native clon d          301 AGCTACCCTTACAACTACTTCGATGTCTGGGGCCAAGGGACCACGGTCAC 350
2dVH-TTCH_N87D_cloned            301 ..................................................... 350
2dVH-TTCH N87D_G53C_cloned       301 ..................................................... 350
2dVH-TTCH_N87D_G54C_cloned       301 ..................................................... 350
2dVH-TTCH N87D_G55C_cloned       301 ..................................................... 350
2dVH-TTCH expected sequence      301 ..................................................... 350
2d12.5 VH native hybridoma       295 .....................................A..... 344

                                         360       370       380       390       400
                                     ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned          351 CGTCTCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCT 400
2dVH-TTCH_N87D_cloned            351 ..................................................... 400
2dVH-TTCH N87D_G53C_cloned       351 ..................................................... 400
2dVH-TTCH_N87D_G54C_cloned       351 ..................................................... 400
2dVH-TTCH N87D_G55C_cloned       351 ..................................................... 400
2dVH-TTCH expected sequence      351 ..................................................... 400
2d12.5 VH native hybridoma       345 .......T..                                          354

                                         410       420       430       440       450
                                     ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned          401 CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG 450
2dVH-TTCH_N87D_cloned            401 ..................................................... 450
2dVH-TTCH N87D_G53C_cloned       401 ..................................................... 450
2dVH-TTCH_N87D_G54C_cloned       401 ..................................................... 450
2dVH-TTCH N87D_G55C_cloned       401 ..................................................... 450
2dVH-TTCH expected sequence      401 ..................................................... 450
2d12.5 VH native hybridoma

                                         460       470       480       490       500
                                     ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned          451 GACTACTTCCCCGAACCGGTGACGGTGTCTTGGAACTCAGGCGCCCTGAC 500
2dVH-TTCH_N87D_cloned            451 ..................................................... 500
2dVH-TTCH N87D_G53C_cloned       451 ..................................................... 500
2dVH-TTCH_N87D_G54C_cloned       451 ..................................................... 500
2dVH-TTCH N87D_G55C_cloned       451 ..................................................... 500
2dVH-TTCH expected sequence      451 ..........................G.......................... 500
2d12.5 VH native hybridoma

                                         510       520       530       540       550
                                     ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned          501 CAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT 550
2dVH-TTCH_N87D_cloned            501 ..................................................... 550
2dVH-TTCH N87D_G53C_cloned       501 ..................................................... 550
2dVH-TTCH_N87D_G54C_cloned       501 ..................................................... 550
2dVH-TTCH N87D_G55C_cloned       501 ..................................................... 550
2dVH-TTCH expected sequence      501 ..................................................... 550
2d12.5 VH native hybridoma

                                         560       570       580       590       600
                                     ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_native cloned          551 CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC 60
2dVH-TTCH_N87D_cloned            551 ..................................................... 60
2dVH-TTCH N87D_G53C_cloned       551 ..................................................... 60
2dVH-TTCH_N87D_G54C_cloned       551 ..................................................... 60
2dVH-TTCH N87D_G55C_cloned       551 ..................................................... 60
2dVH-TTCH expected sequence      551 ..................................................... 60
2d12.5 VH native hybridoma

                                         610       620       630       640       650
                                     ....|....|....|....|....|....|....|....|....|....|
2dVH-TTCH_nativ  cloned          601 TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA 6
2dVH-TTCH_N87D_cloned            601 ..................................................... 6
```

FIG.9C

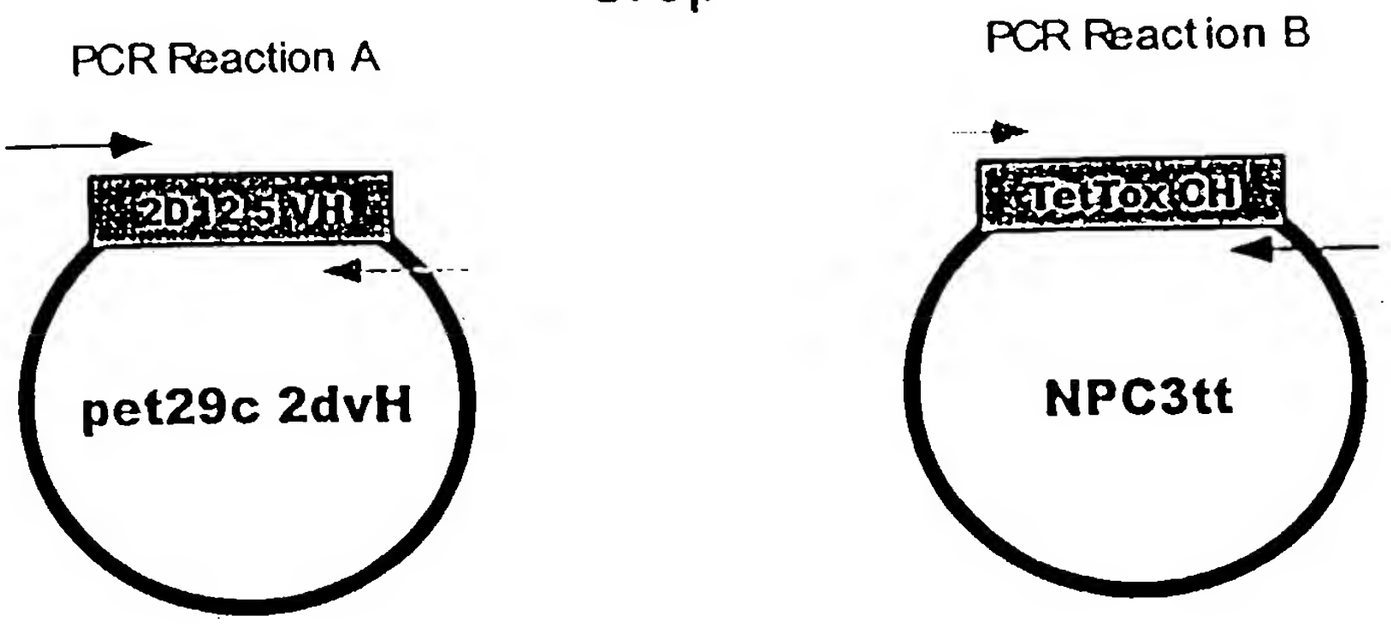
Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

```
2dVH-TTCH N87D_G53C_cloned    601 ..................................................  650
2dVH-TTCH_N87D_G54C_clon d    601 ..................................................  650
2dVH-TTCH N87D_G55C_cloned    601 ..................................................  650
2dVH-TTCH expected sequence   601 ..................................................  650
2d12.5 VH native hybridoma

                                                660       670       680
                                          ....|....|....|....|....|....|.
2dVH-TTCH_native cloned       651 AGCAGAGCCCAAATCTTGTGACAAAATCTAGA 681
2dVH-TTCH_N87D_cloned         651 ................................. 681
2dVH-TTCH N87D_G53C_cloned    651 ................................. 681
2dVH-TTCH_N87D_G54C_cloned    651 ................................. 681
2dVH-TTCH N87D_G55C_cloned    651 ................................. 681
2dVH-TTCH expected sequence   651 ................................. 681
2d12.5 VH native hybridoma
```

Strategy for Assembly of Chimeric 2D12.5 Heavy Chain

Step 1



PCR Products

A [redacted]

B [redacted]

PCR cycle - 2DTTover
1 - 94° C 10 min
2 - 94° C 1 min
3 - 62° C 1 min
4 - 72° C 2 min
5 - 25x to step 2
6 - 72° C 5 min
7 - 4° C 18 hr
8 - END
Taq added before step 2

Example reaction

d2H2O	63ul
10x buffer	10uL
25mM MgCl2	8uL
2mM dNTP	10uL
Template 1 (1ng/ uL)	1uL
Top Primer 25pmol/ uL	4uL
Bottom Primer 25pmol/ uL	4uL
Taq Polymerase	0.5uL

Primers

→ VH2D5' BglII
GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

← VH2DTTMi
GCCCTTGGTGGAGGCTGCGGAGACGGTGACC-GTGG

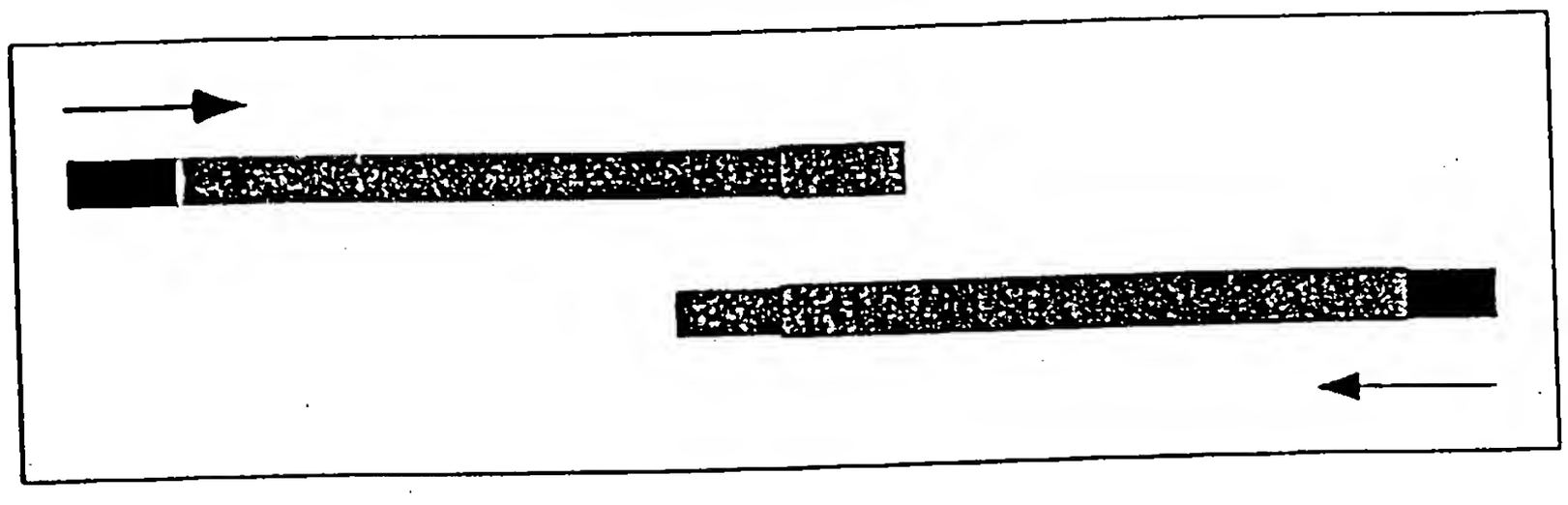
→ CHTT2DMi
CCACGGTCACCGTCTCCGCAGCCTCCAC-CAAGGGC

← TTCH3' Xba
CGATCTAGATTTGTCACAAGATTTGGGCTCTGC

FIG. 10B

Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 2

PCR Reaction



PCR cycle - 2DTTVent
1 - 95°C 10 min
2 - 94°C 1 min
3 - 60°C 1 min
4 - 75°C 2 min
5 - 4x to step 2
6 - 94°C 1 min
7 - 63°C 1 min
8 - 75°C 2 min
9 - 25x to step 6
10 - 72°C 5 min
11 - 4°C 18 hr
12 - END
Vent added before step 2
Primers added before step 6

Primers

→ VH2D5' BglII
GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

← TTCH3' Xba
CGATCTAGATTTGTCACAAGATTTGGGCTCTGC

Example reaction

d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template 1(1ng/ uL)	1uL
Template 2(1ng/ uL)	1uL
Top Primer 25pmol/ uL	4uL
Bottom Primer 25pmol/ uL	4uL
Vent Polymerase	0.5uL

PCR Assembly Product

FIG. 10C

Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 3

Desired PCR Assembly Product

Restriction Digest PCR Product with Bgl II & Xba I

Ligate Restriction Digested PCR Product into pMTBipV5His (S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

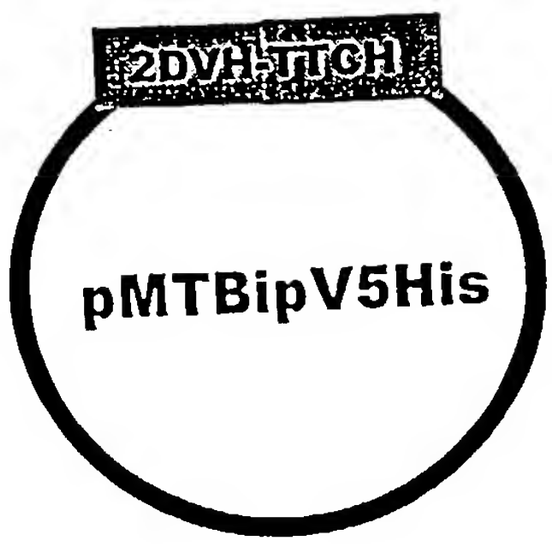
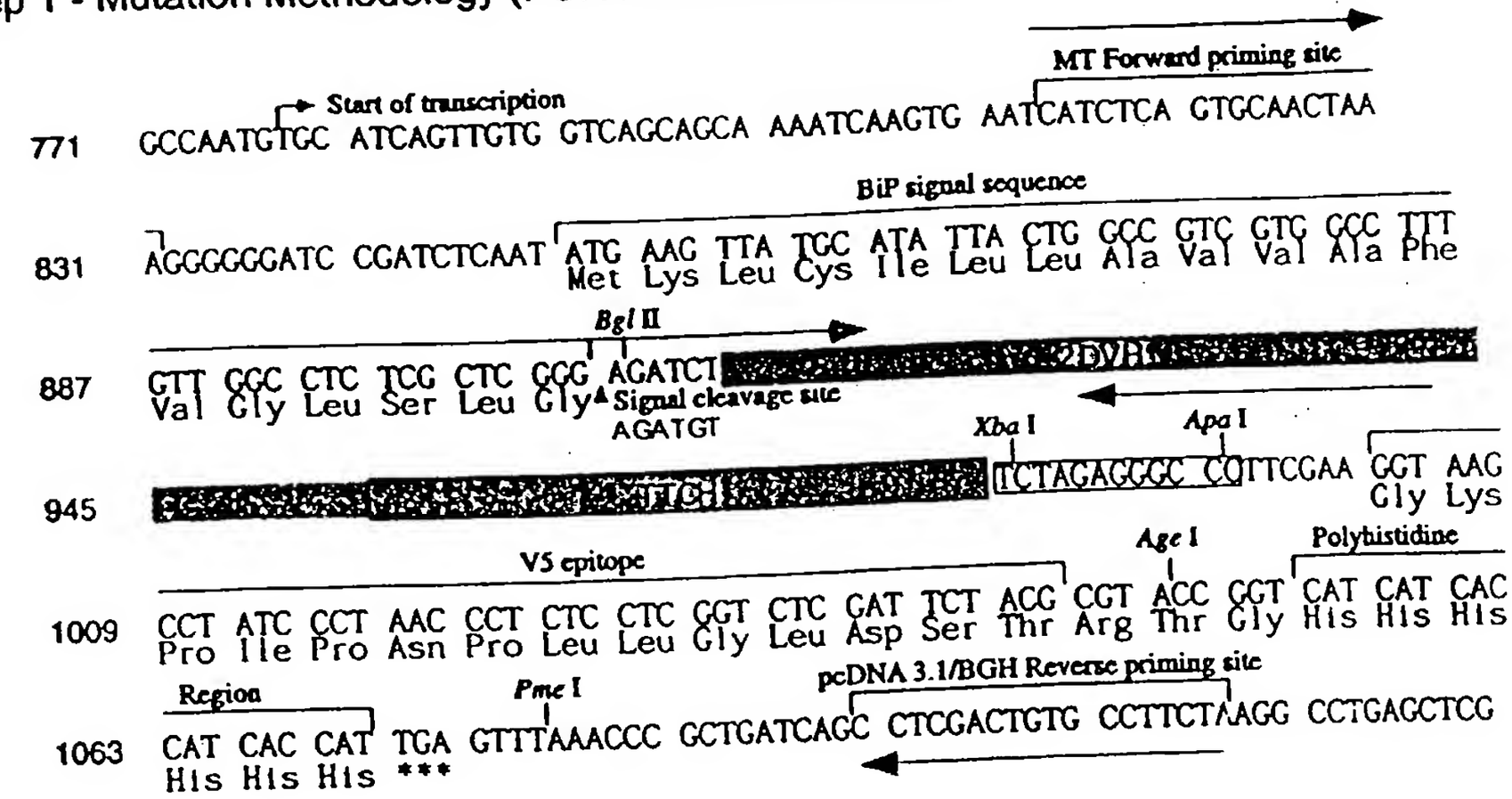


FIG. 10D

Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



1st Set of PCR Reactions Producta A and B)

CATCTCAGTGCAACTAAA	MTforward
CATGGCTGTGTCATCAGCTTGCAGACTGTTC	2dvhN87D_pMTBip
	OR
CGTGCCTCCACAACTCCATATCAC	G53C noncoding 2dG53c_pMT
	OR
CCGTGCCACAACCACTCCATATC	G54C noncoding 2dG54c_pMT
	OR
CCGTGCATCCACCACTCCATATC	G55C noncoding 2dG55c_pMT

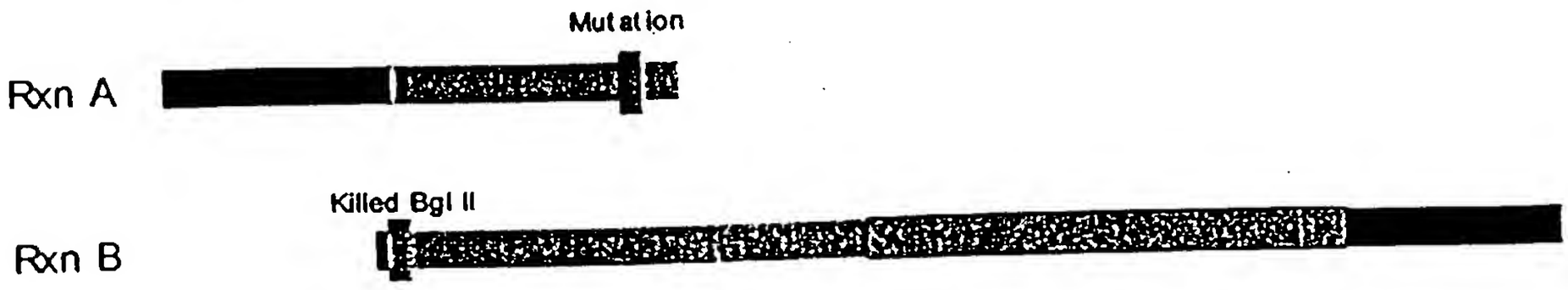
GCTCGGGAGATGTGTGAAGCTG	2dvhKBglII_pMTBip
TAGAAGGCACAGTCGAGG	BGHreverse

FIG. 10E

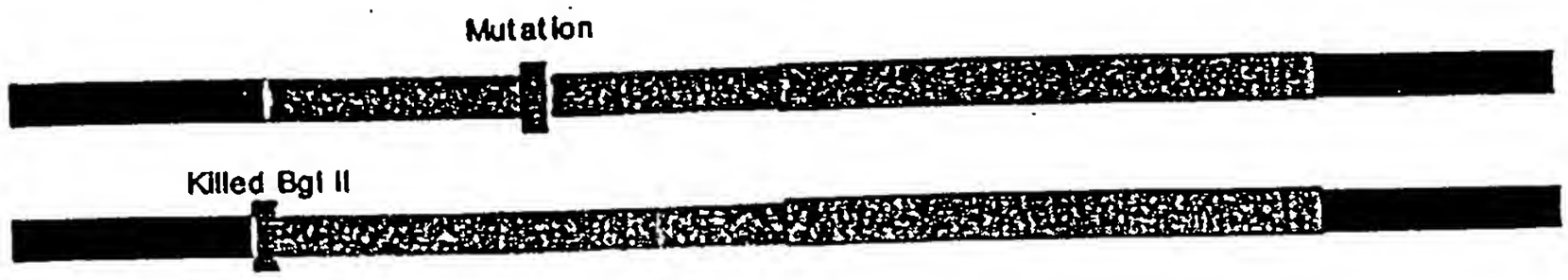
Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

2nd PCR Reaction (Mix Products of reaction A and B)

1) Extend
2) Amplify with outer primers (MTforward and BGHreverse)



2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1

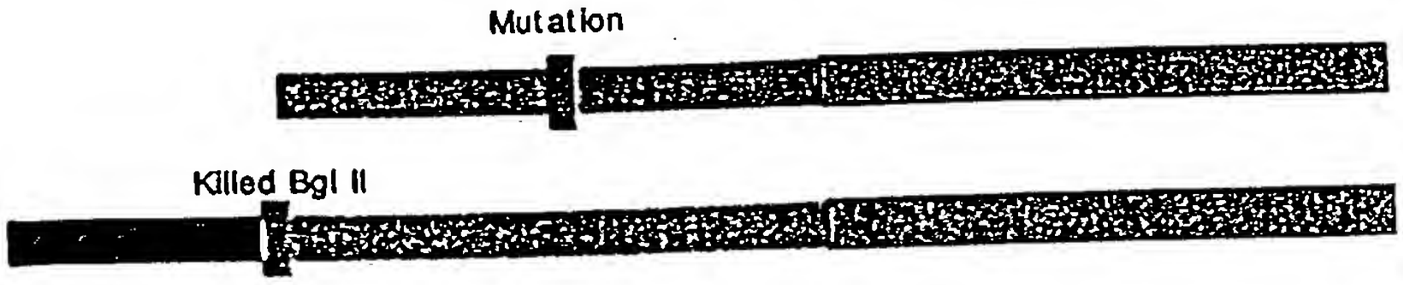


FIG. 10F

Step 1 - PCR Reaction MT-VENT

PCR cycle - MT-VENT
1 - 95° C 10 min
2 - 94° C 1 min
3 - 50° C 1 min
4 - 75° C 2 min
5 - 24x to step 2
6 - 75° C 5 min
7 - 4° C 18 hr
8 - END
VENT added before step 2
Primers added before step 1

Example reaction

d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Vent Polymerase	0.5uL

Step 2 - PCR Reaction VHMUTTAQ

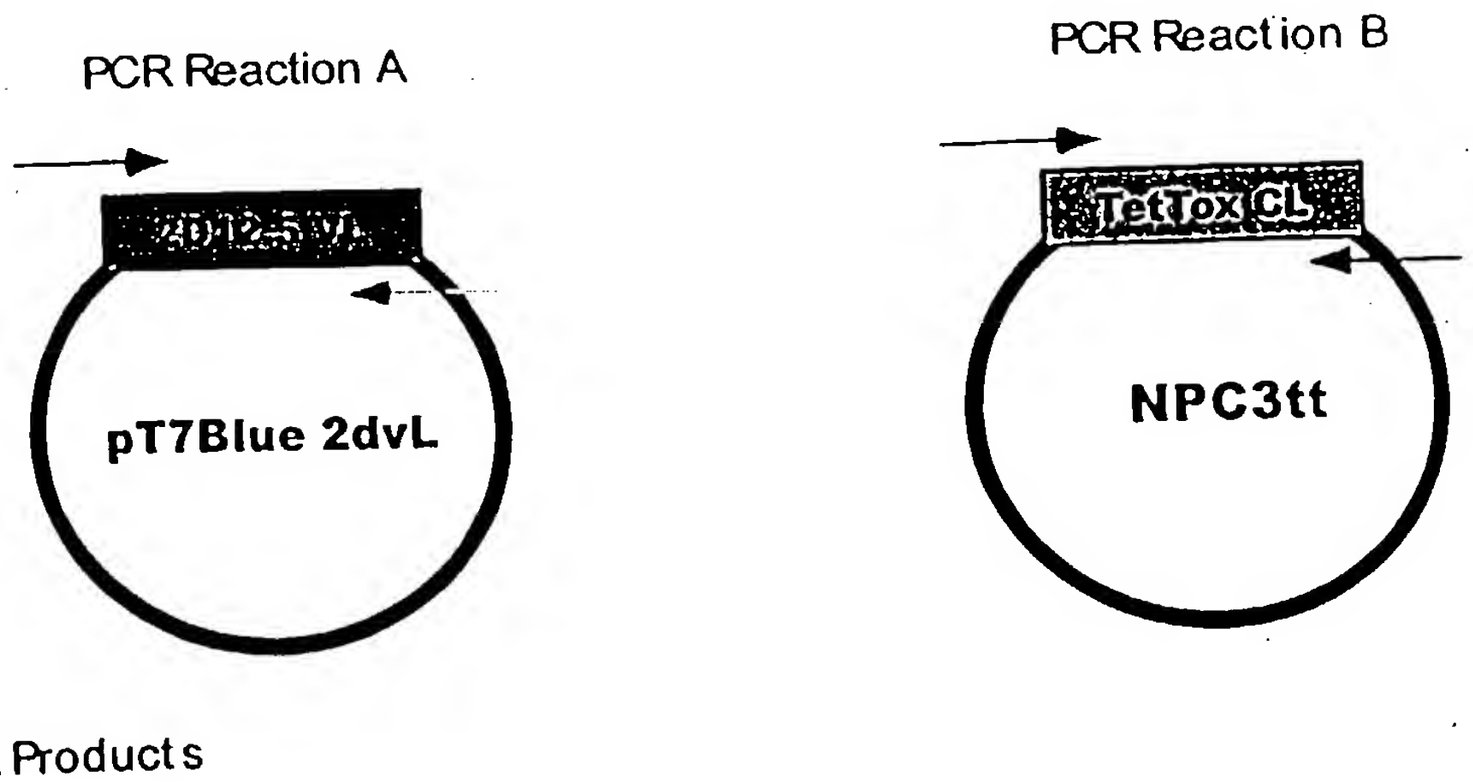
PCR cycle - VHMUTTAQ
1 - 95° C 10 min
2 - 94° C 1 min
3 - 68° C 1 min
4 - 72° C 2 min
5 - 4x to step 2
6 - 94° C 1 min
7 - 50° C 1 min
8 - 72° C 2 min
9 - 24x to step 6
10 - 72° C 5 min
11 - 4° C 18 hr
12 - END
Taq added before step 2
Primers added before step 6

Example reaction

d2H2O	61ul
10x buffer	10uL
25mM MgCl2	8uL
2mM dNTP	10uL
Template 1 (1ng/uL)	1uL
Template 2 (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Taq Polymerase	0.5uL

FIG. 11A

Strategy for Assembly of Chimeric 2D12.5 Light Chain
Step 1



Primers

2dVL5'Bgl

GAAGATCTGCTGTTGTGACTCAGGAATCT

2dVLMiBot

AGATGGTGCAGCCACAGTTCGGCTTAGGA-
CAGTCAGTTTGGT

2dVLMiTop

ACCAAACTGACTGTCCTAAGCCGAACTGT-
GGCTGCACCATCT

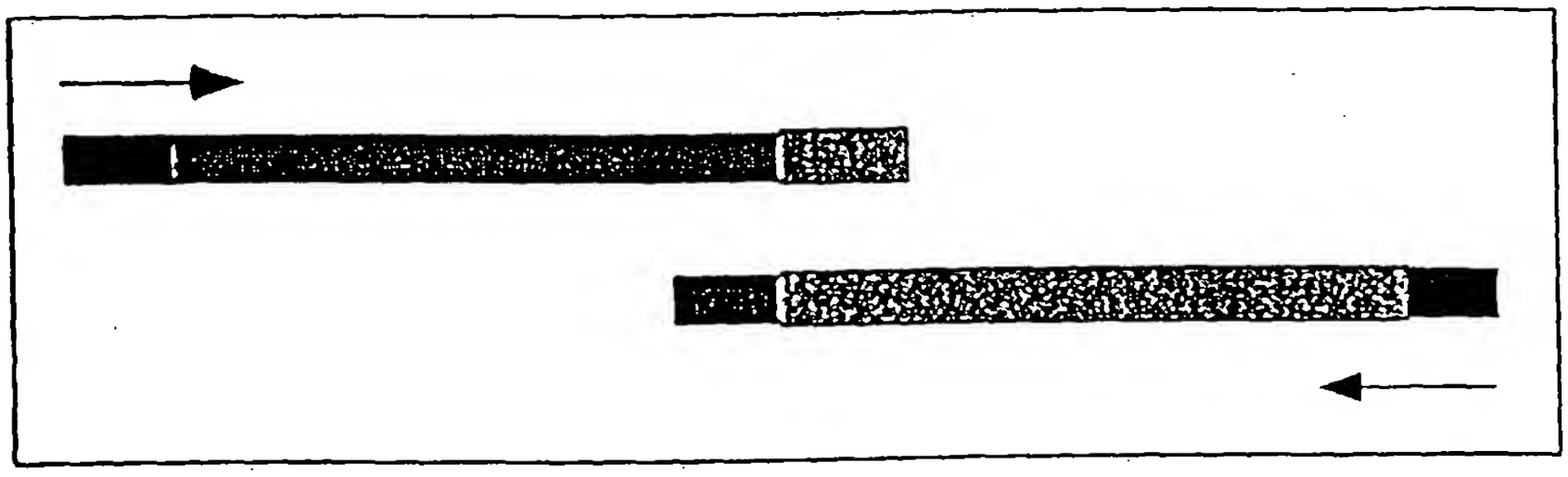
TTCL3' Xba

CGATCTAGAATTAACACTCTCCCCTG

FIG. 11B

Strategy for Assembly of Chimeric 2D12.5 Light Chain
Step 2

PCR Reaction



Primers

→ 2dVL5'Bgl

GAAGATCTGCTGTTGTGACTCAGGAATCT

← TTCL3' Xba

CGATCTAGAATTAACACTCTCCCCTG

PCR Assembly Product

FIG. 11C

Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 3

Desired PCR Assembly Product

Restriction Digest PCR Product with Bgl II & Xba I

Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

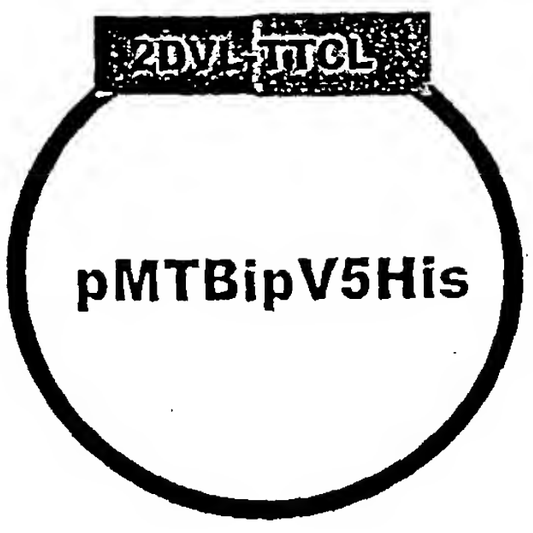
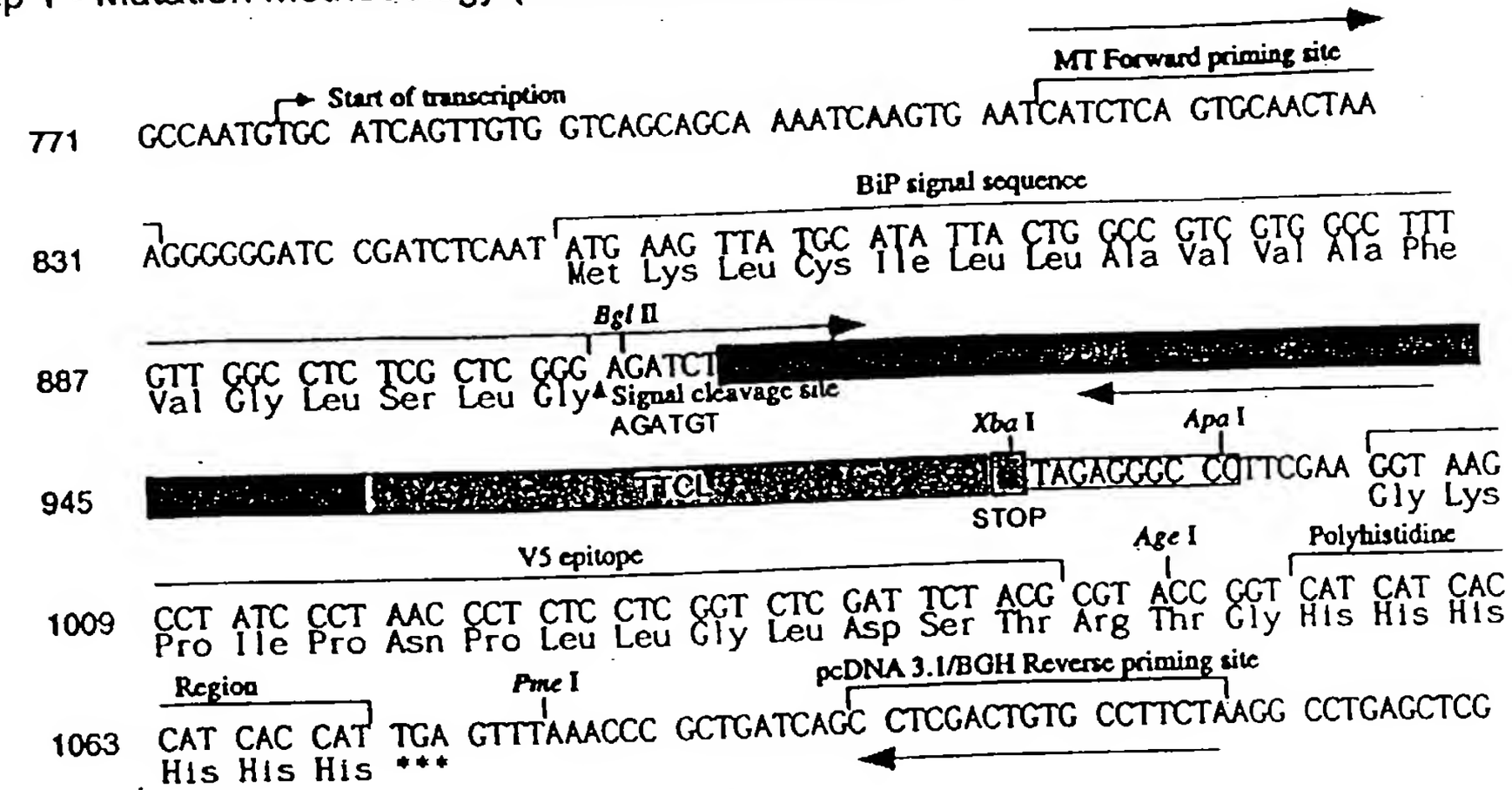


FIG. 11D

Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



1st Set of PCR Reactions Producta A and B)

CATCTCAGTGCAACTAAA	MTforward
CACCTATTAGACCAGTGAATAAATG	2dvL_S48G_pMTBip OR
CGGTTATTACAACCACCTATTAGACCAGT-GAATAAATG	S48G_N53C_pMTBip

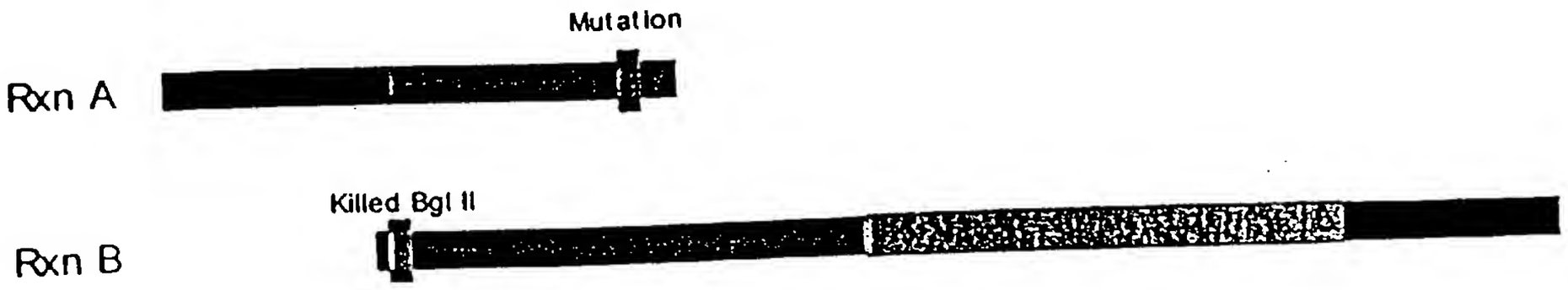
GCTCGGGAGATGTGCTGTTGTG	2dvLKBglII_pMTBip
TAGAAGGCACAGTCGAGG	BGHreverse

FIG. 11E

Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

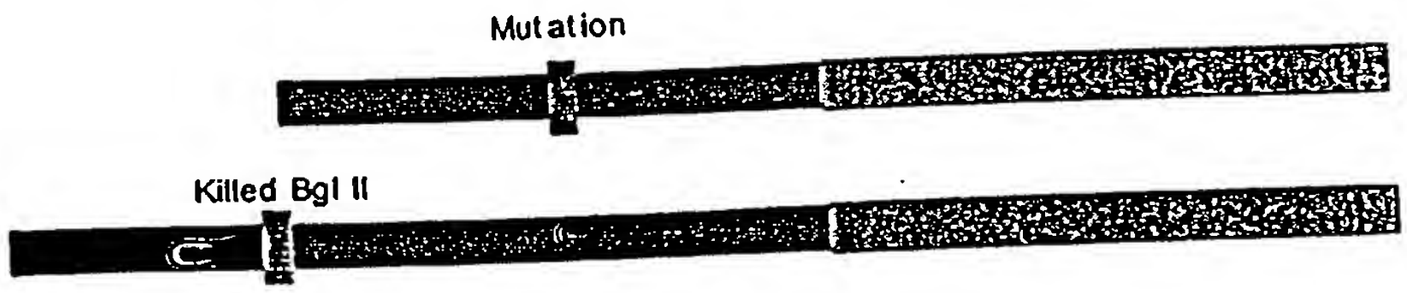
2nd PCR Reaction Mix Products of reaction B)

1) Extend
2) Amplify with outer primers (MTforward and BGHreverse)

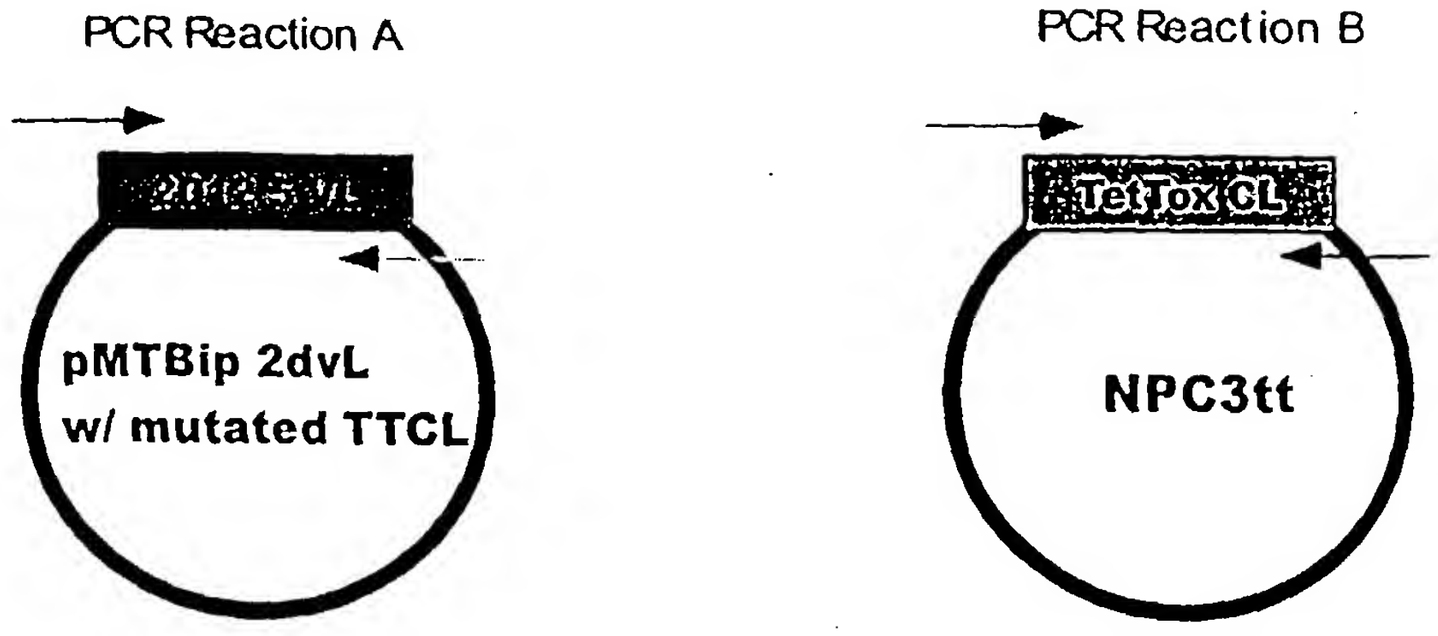


2nd PCR Reaction Products (Mixture - 2 Products of equal size)

Restriction Digest PCR Product Mixture with BglII and Xba1



Strategy for Assembly of Chimeric 2D12.5 Light Chain
Step 4



PCR Products

A

B

PCR cycle - 2DTTover
1 - 94° C 10 min
2 - 94° C 1 min
3 - 62° C 1 min
4 - 72° C 2 min
5 - 25x to step 2
6 - 72° C 5 min
7 - 4° C 18 hr
8 - END
Taq added before step 2

Example reaction		
d2H2O		63ul
10x buffer		10uL
25mM MgCl2	8uL	
2mM dNTP		10uL
Template 1 (1ng/uL)	1uL	
Top Primer 25pmol/uL		4uL
Bottom Primer 25pmol/uL	4uL	
Taq Polymerase		0.5uL

Primers

→ Mtforward
CATCTCAGTGCAACTAAA

→ 2dVLMiTop
ACCAAACTGACTGTCCTAAGCCGAACT-
GTGGCTGCACCATCT

← 2dVLMiBot
AGATGGTGCAGCCACAGTTCGGCTTAGGA-
CAGTCAGTTTGGT

← TTCL3' Xba
CGATCTAGAATTAACACTCTCCCCTG

Assembled Vectors for Transfection in S2 Cells

Each of the following has been cotransfected with the native light chain:

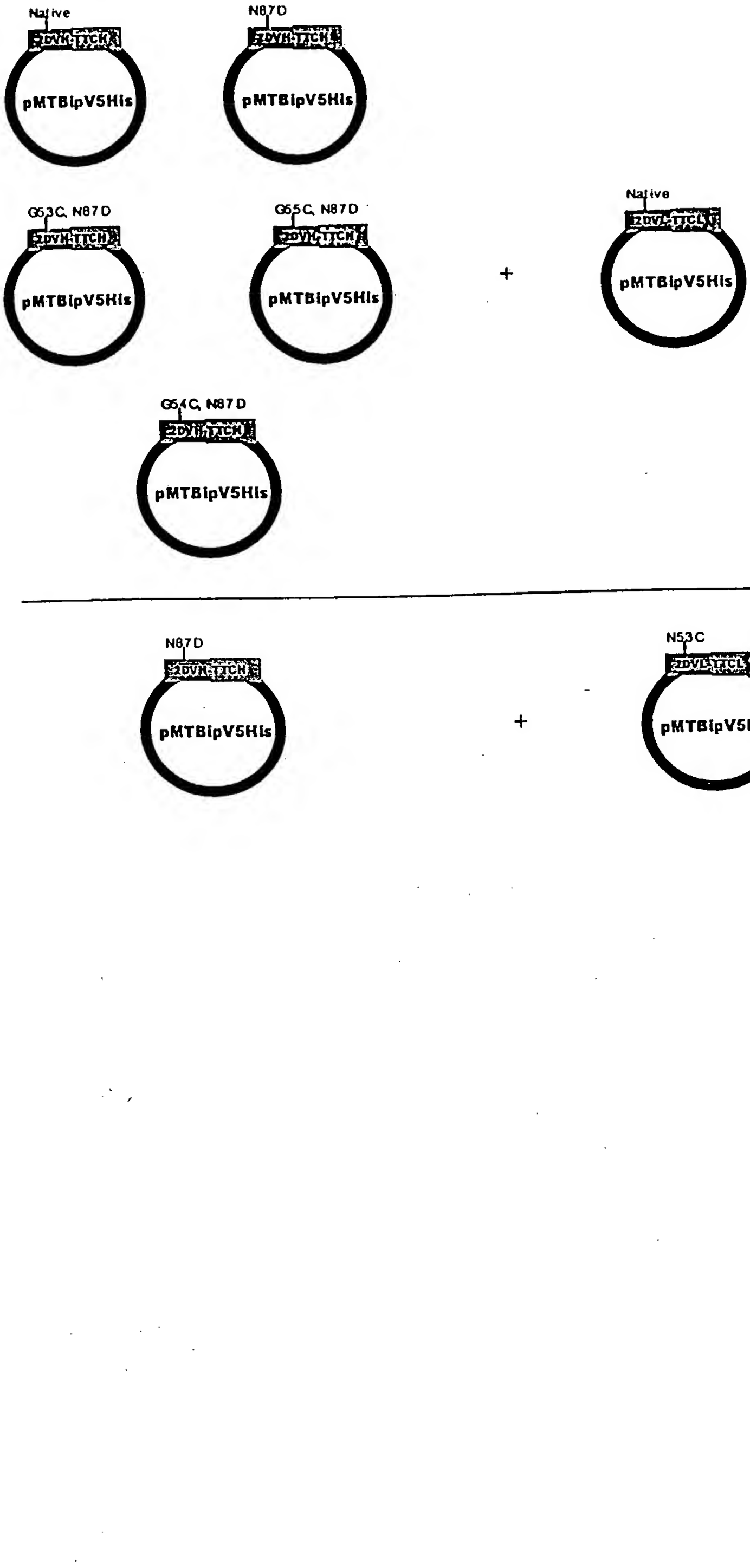
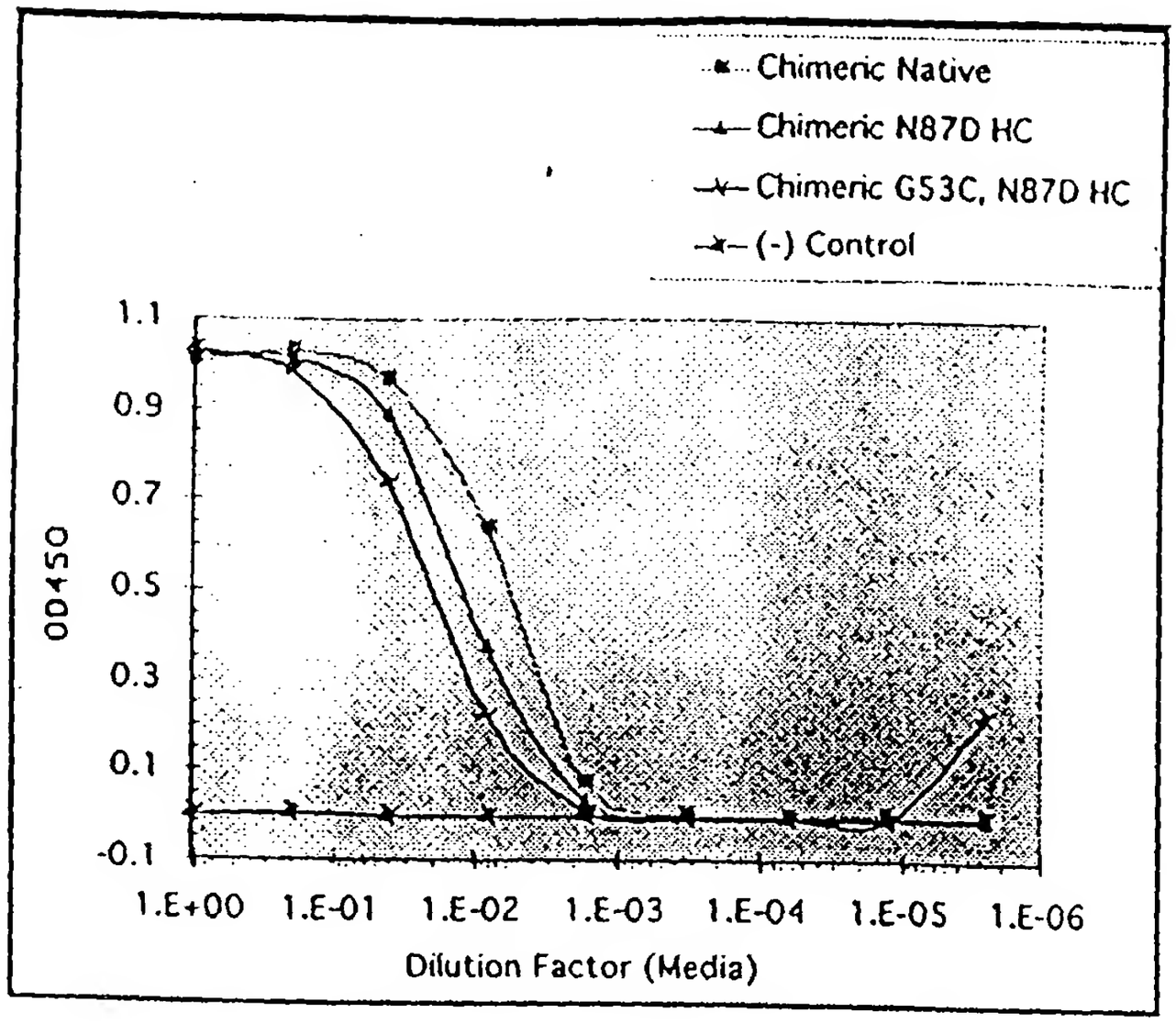


FIG. 12


Chimeric Native
Chimeric N87D HC
Chimeric G53C, N87D HC
(-) Control
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
00450
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
1.E-06
Dilution Factor (Media)

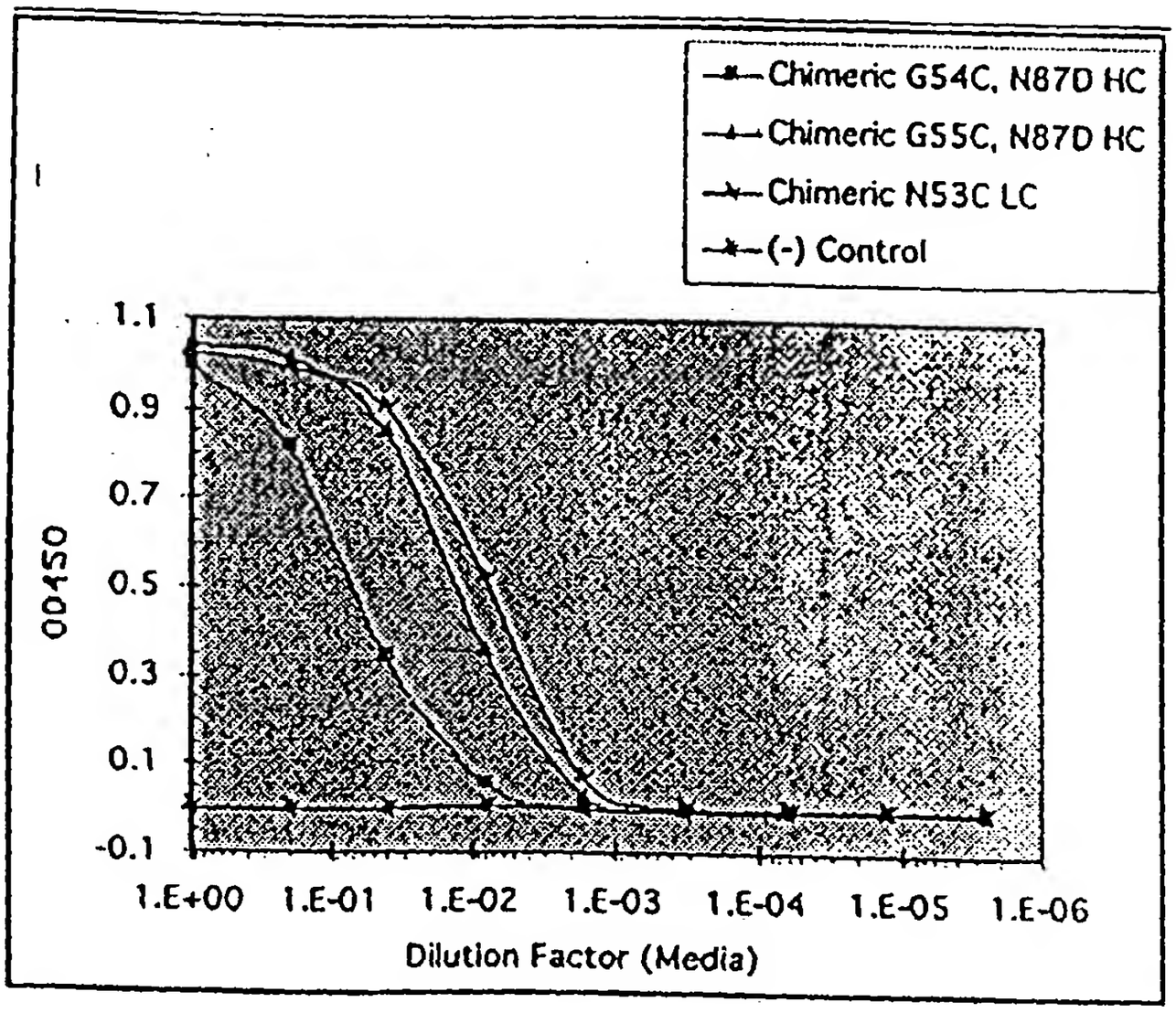

Chimeric G54C, N87D HC
Chimeric G55C, N87D HC
Chimeric N53C LC
(-) Control
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
00450
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
1.E-06
Dilution Factor (Media)

FIG. 13

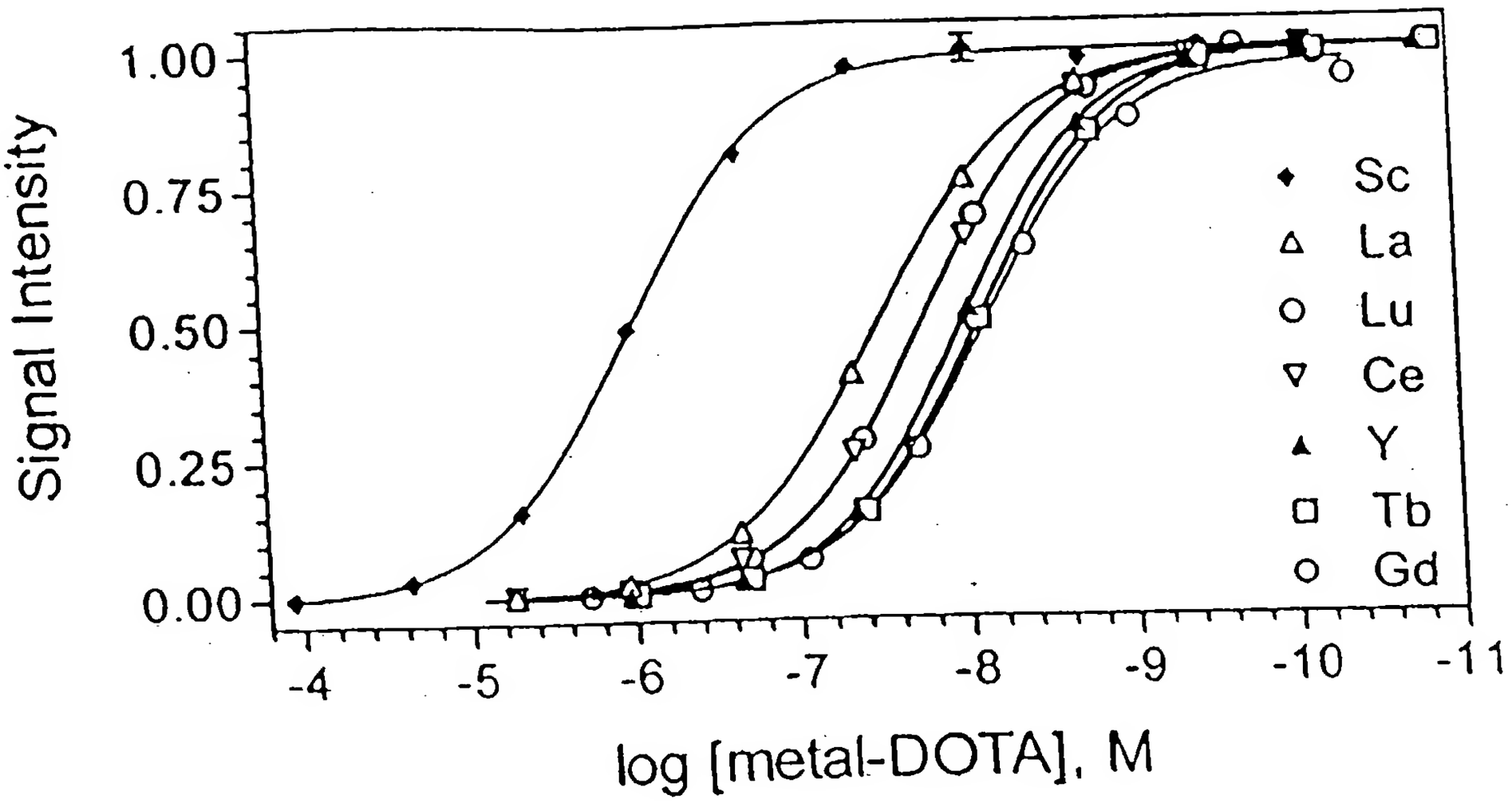

Signal Intensity
1.00
0.75
0.50
0.25
0.00
-4
-5
-6
-7
-8
-9
-10
-11
log [metal-DOTA], M
Sc
La
Lu
Ce
Y
Tb
Gd

FIG. 14

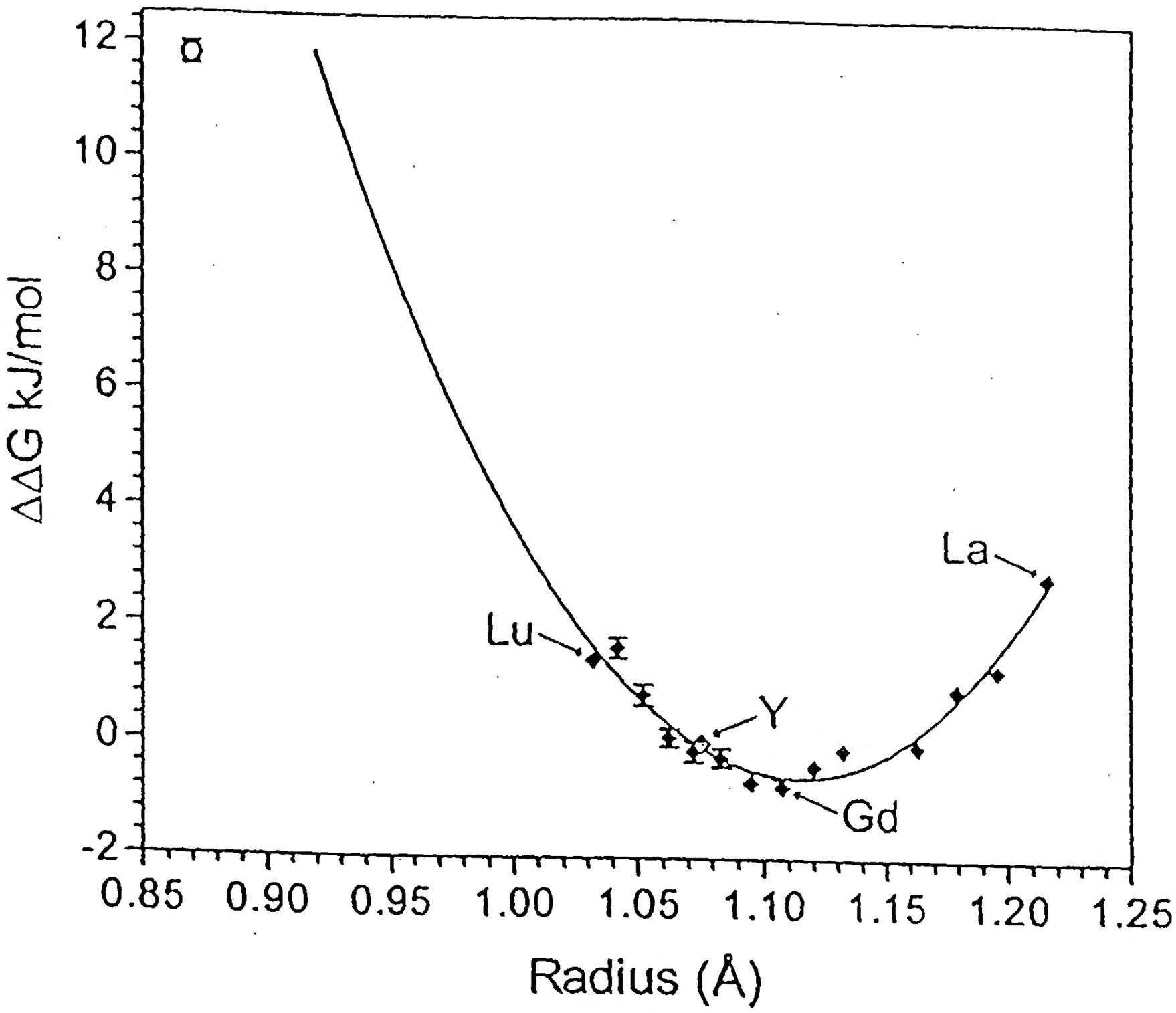

α
12
10
8
6
4
2
0
-2
ΔΔG kJ/mol
La
Lu
Y
Gd
0.85
0.90
0.95
1.00
1.05
1.10
1.15
1.20
1.25
Radius (Å)

FIG. 15

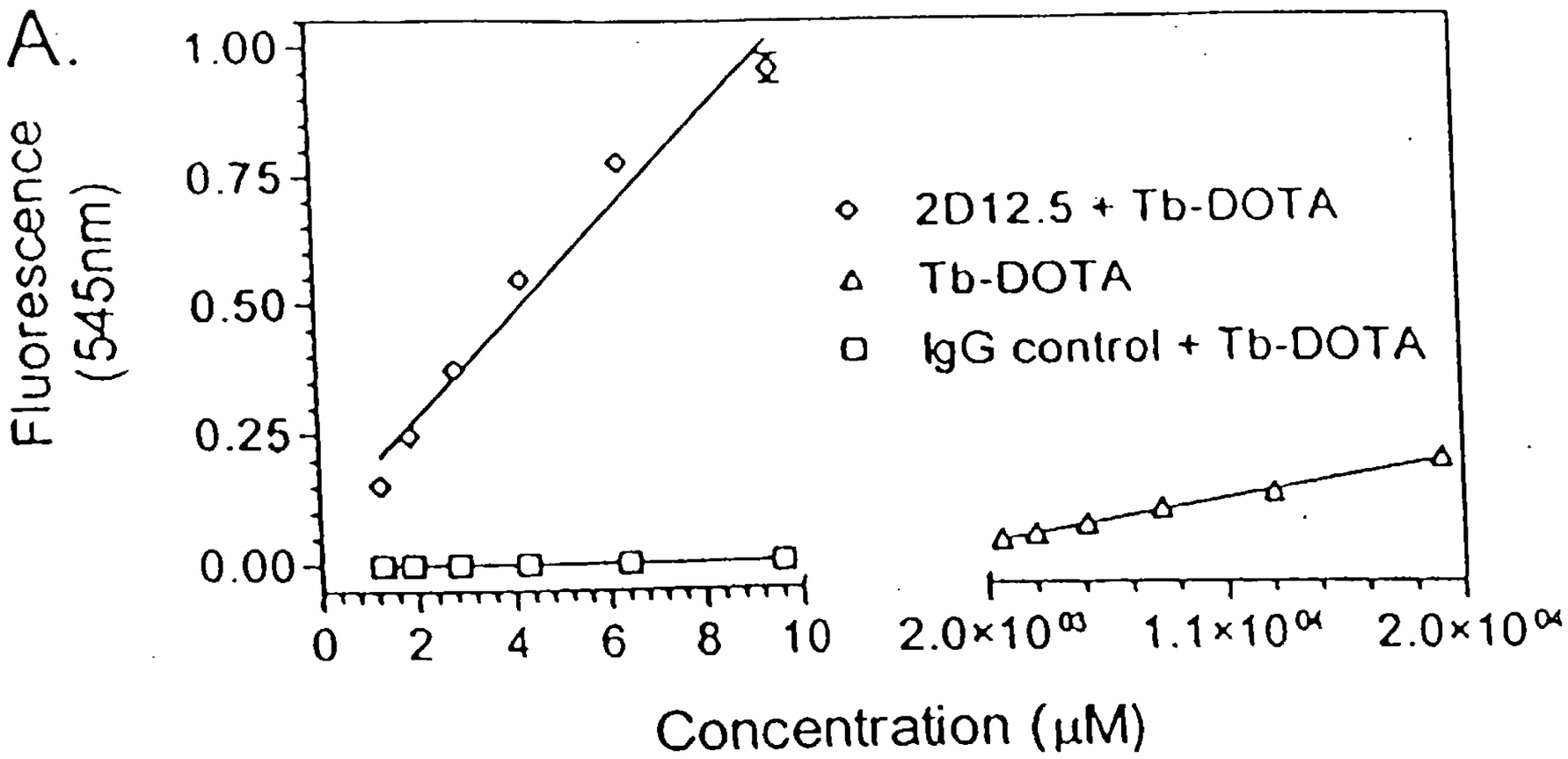

A.
Fluorescence (545nm)
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
2.0×10⁰³
1.1×10⁰⁴
2.0×10⁰⁴
2D12.5 + Tb-DOTA
Tb-DOTA
IgG control + Tb-DOTA
Concentration (μM)

FIG. 16

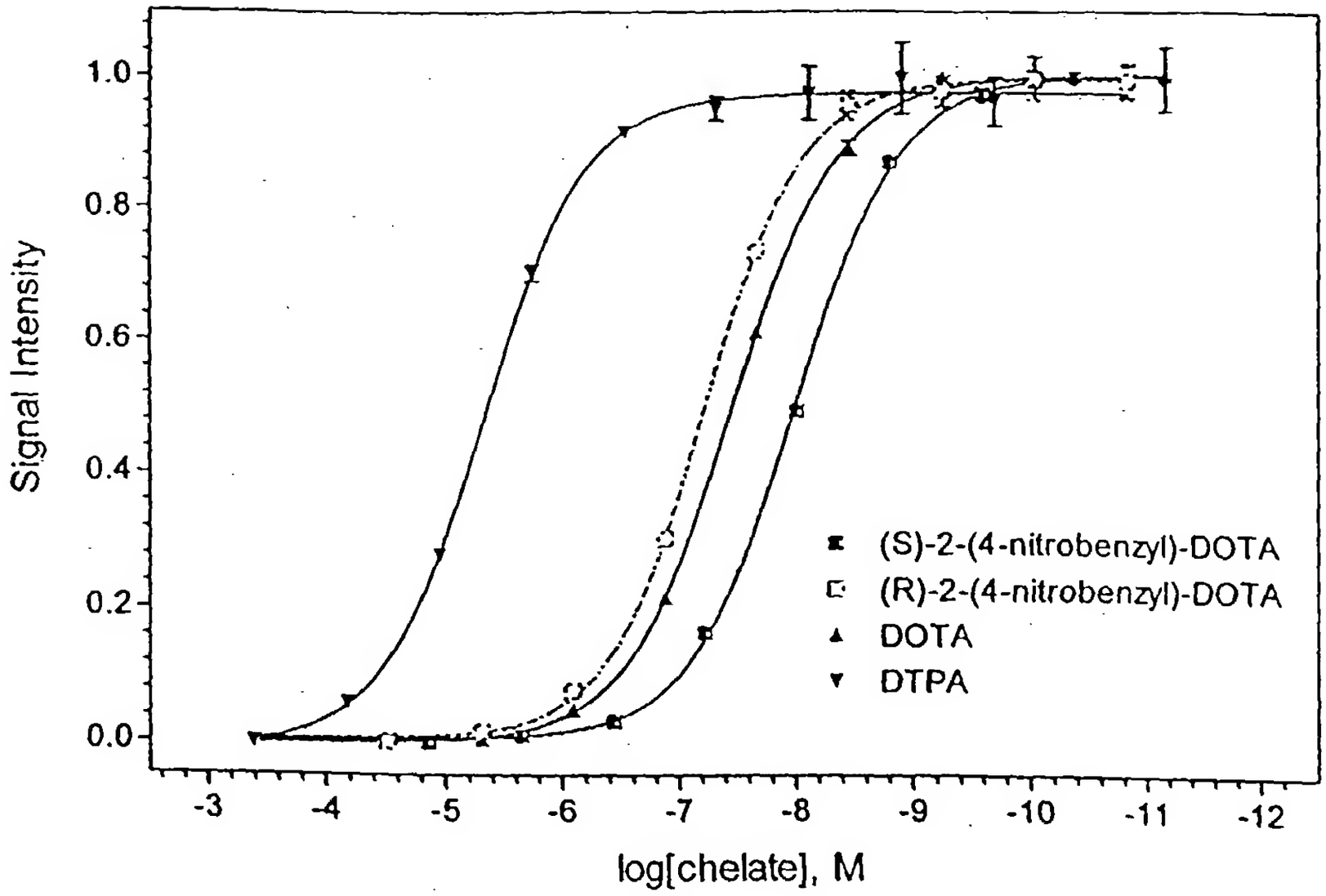

Signal Intensity
1.0
0.8
0.6
0.4
0.2
0.0
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
log[chelate], M
(S)-2-(4-nitrobenzyl)-DOTA
(R)-2-(4-nitrobenzyl)-DOTA
DOTA
DTPA

FIG. 17

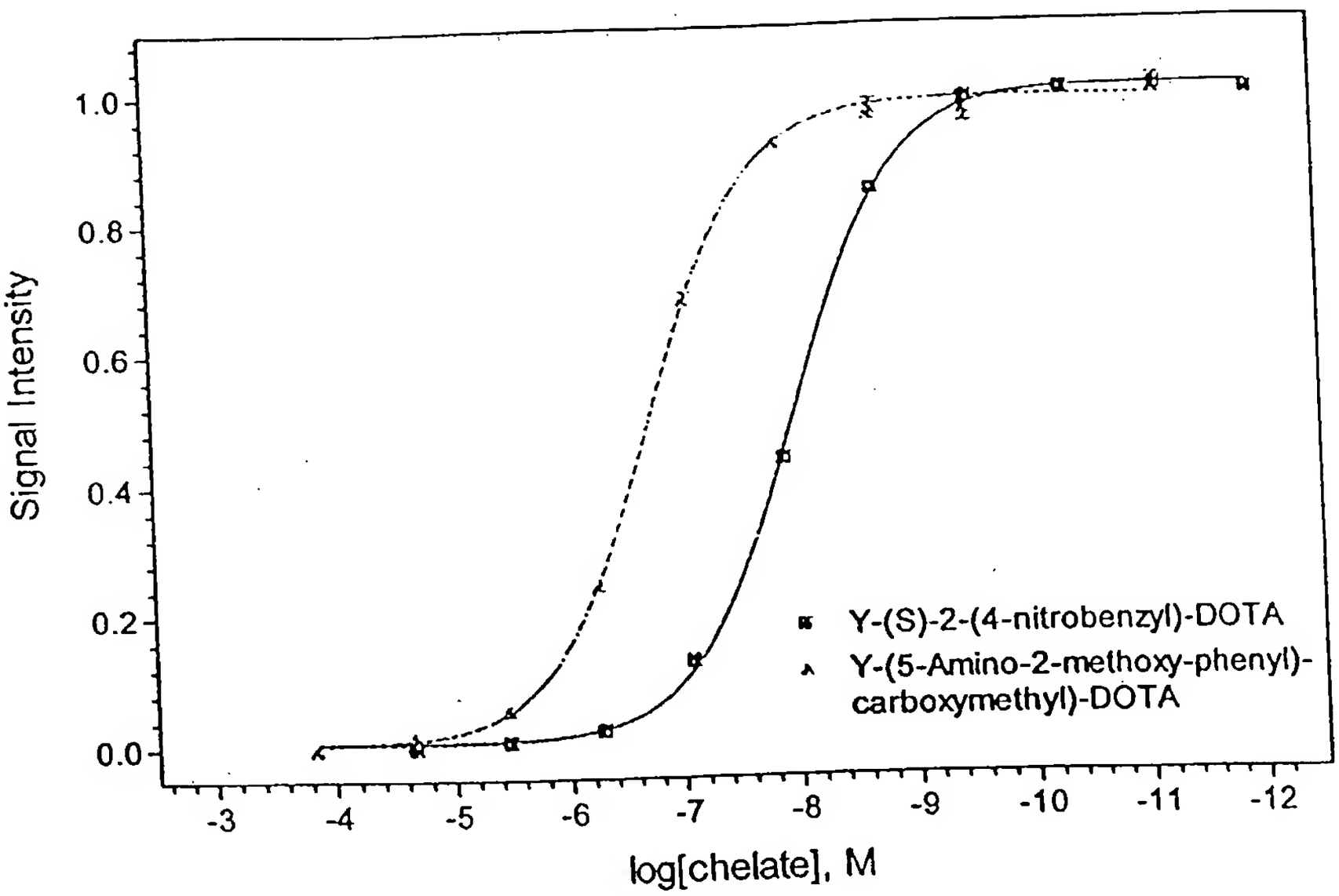
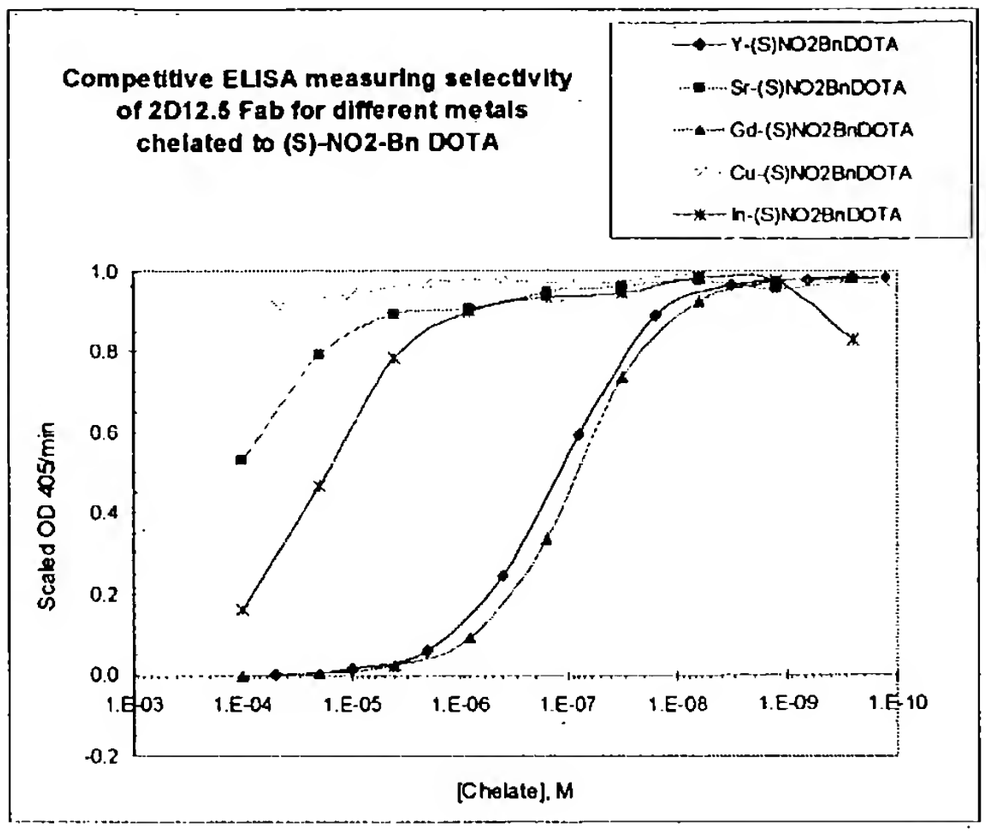
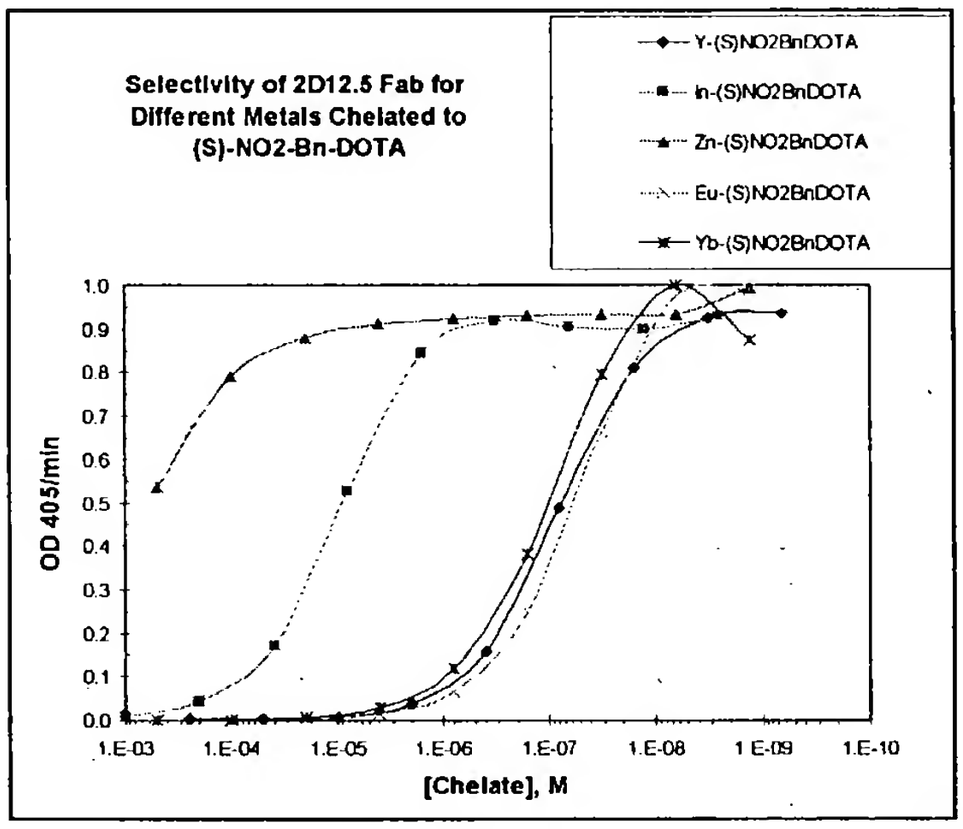

Signal Intensity
1.0
0.8
0.6
0.4
0.2
0.0
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
log[chelate], M
Y-(S)-2-(4-nitrobenzyl)-DOTA
Y-(5-Amino-2-methoxy-phenyl)-carboxymethyl)-DOTA

Figure 18


Competitive ELISA measuring selectivity of 2D12.5 Fab for different metals chelated to (S)-NO2-Bn DOTA
Y-(S)NO2BnDOTA
Sr-(S)NO2BnDOTA
Gd-(S)NO2BnDOTA
Cu-(S)NO2BnDOTA
In-(S)NO2BnDOTA
Scaled OD 405/min
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
1.E-03
1.E-04
1.E-05
1.E-06
1.E-07
1.E-08
1.E-09
1.E-10
[Chelate], M


Selectivity of 2D12.5 Fab for Different Metals Chelated to (S)-NO2-Bn-DOTA
Y-(S)NO2BnDOTA
In-(S)NO2BnDOTA
Zn-(S)NO2BnDOTA
Eu-(S)NO2BnDOTA
Yb-(S)NO2BnDOTA
OD 405/min
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1.E-03
1.E-04
1.E-05
1.E-06
1.E-07
1.E-08
1 E-09
1.E-10
[Chelate], M

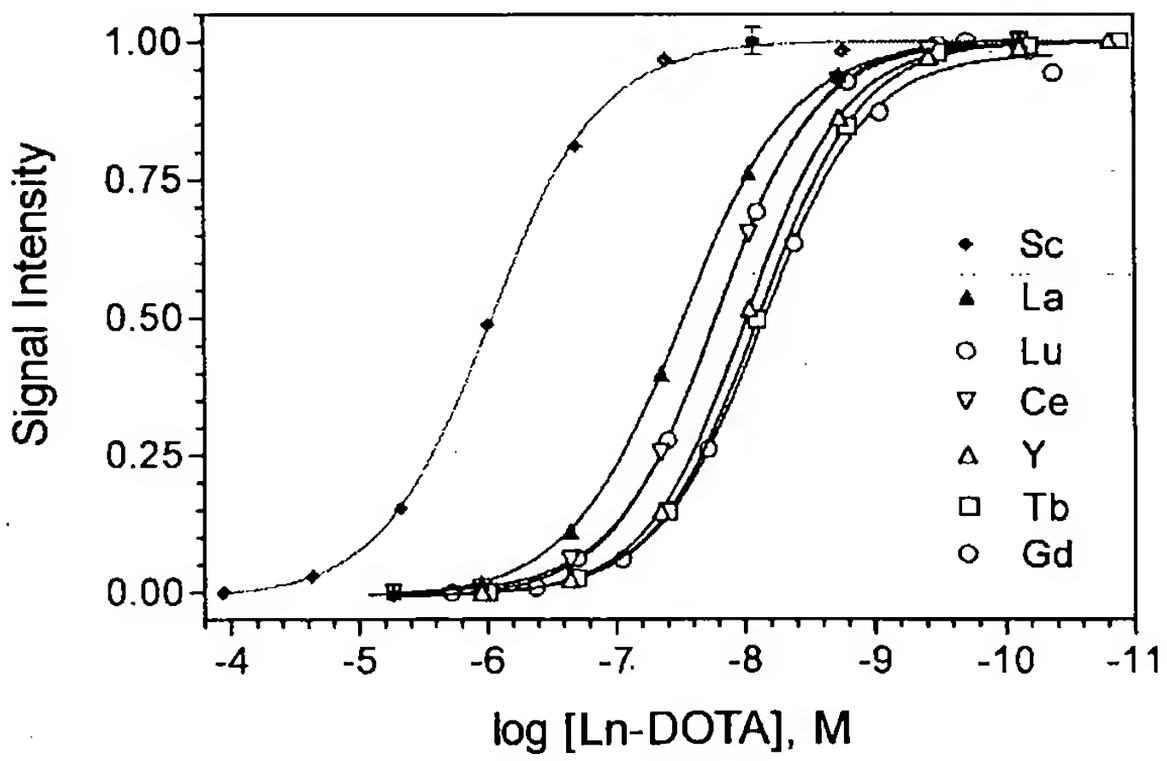
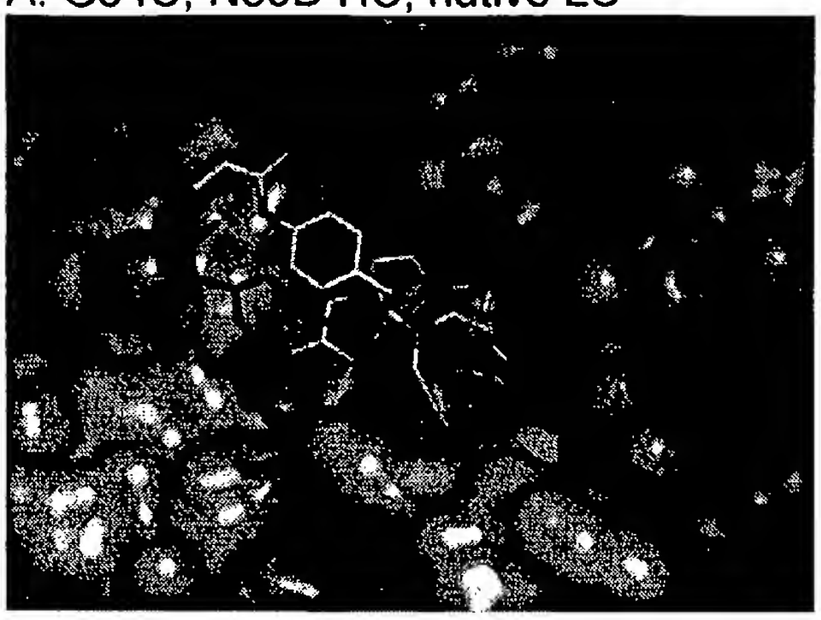

Signal Intensity
1.00
0.75
0.50
0.25
0.00
-4
-5
-6
-7
-8
-9
-10
-11
log [Ln-DOTA], M
Sc
La
Lu
Ce
Y
Tb
Gd

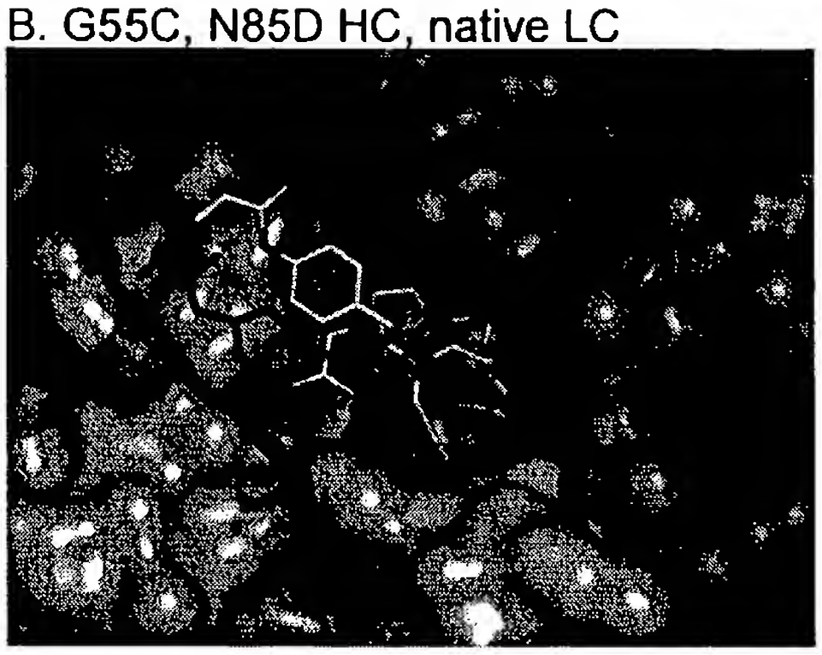
Figure 19

Figure 20

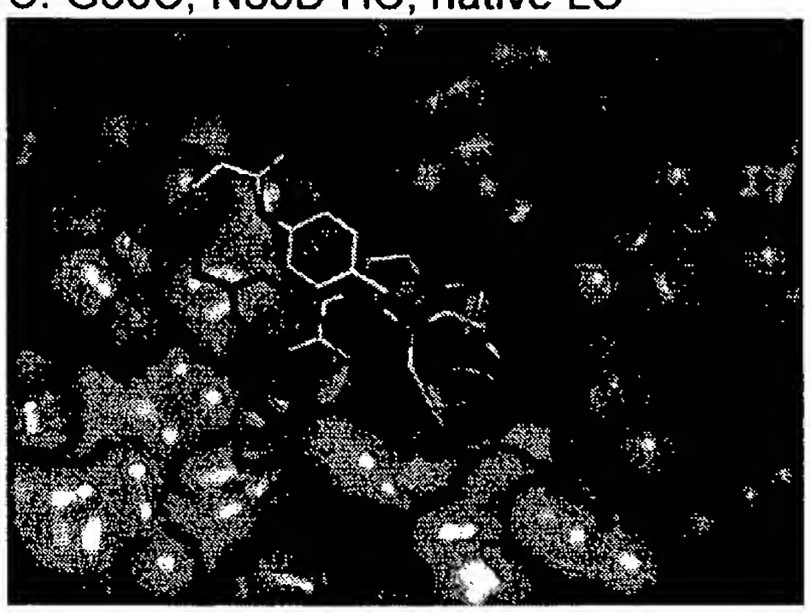
A. G54C, N85D HC, native LC



B. G55C, N85D HC, native LC



C. G56C, N85D HC, native LC



D. N85D HC, N53C LC